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The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

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Associate Editors : Kakuji GOTŌ and Yoshikazu SAHASHI.

The Chemical composition of tunny liver oil.

By

Tetuo TOMIYAMA

(Received Sept. 21, 1933)

Introduction.

In regard to the fatty acids of aquatic animal oils, much study has been made on their body fats, but comparatively little done on their liver oils. Concerning the study of the body fats of tunny (Thunnus orientalis) there is I. Okada's work, but no researches have been done yet on the fatty acids of its liver oil. Again, with reference to the vitamin-A of tunny liver oil, H. Sekine's work came to our attention years ago, but no determination has yet been made regarding its potency. Lately, Y. Kawakami has estimated by color reaction the quantities of vitamin-A contained in the liver oils of many kinds of fish, and reported that tunny liver oil contained 213.6 units, while cod liver oil, 2.7$\sim$7.9 units. The author has made researches on the fatty acids of tunny liver oil and estimated the vitamin-A content by means of biological test. Of course the quantities of these fatty acids vary owing to the factors, such as the seasons of the year, the ages and the sexes of the fish, but it would seem possible to obtain the general inference regarding the contents.

Experiment.

(I) The Preparations of Sample Oil and Mixed Fatty Acids, and Their General Properties.

The oil was obtained by grinding the liver which has passed a day in an ice box after catching the fish in spring time, making it dehydrated powder by mixing with anhydrous Na_2SO_4, then extracting with petroleum ether (b.p.$<50°$). The extract was made to be a constant weight, removing the solvent under the current of CO_2. The oil thus obtained was of liquid form having yellowish brown color, and in winter, it separated a little quantity of solid fat. Its general properties are as shown in Table 1 below. In spring time, the liver contains a large quantity of oil, and the quantity obtained amounted to 26% of the fresh one. As its degree of unsaturation was high and its oxidation during the chemical treatment was considerable, special efforts have been made to perform the following treatments in dried CO_2.

Table 1

Acid value	28.0	Reichert-Meissl value	2.2
Saponification value	177.6	Polenski value	0.5
Ester value	149.6	Specific gravity (15°C.)	0.924
Iodine value (Wijs' method)	175.6	Unsaponifiable matter	1.6%
Acetyl value	31.5	Melting point	28~29°C.
Hehner value	89.8		

The fatty oil was saponified by the usual method for about 40 minutes at 60°C., adding to it alcoholic caustic potash. After eliminating the unsaponifiable matter by ether, the saponified liquid was decomposed by H_2SO_4, and the liberatd fatty acids were transferred into ether and washed several times with water until free from mineral acid; then, they were dehydrated by anhydrous Na_2SO_4 and the solvent was removed under diminished pressure. The mixed fatty acids thus obtained were of red brown color, and at ordinary temperature they were solid. The general properties of mixed fatty acids and of the divisions separated into the liquid and the solid by the lead salt ether method are as given in the following Table 2.

Table 2

	Mixed fatty acid	Liquid fatty acid	Solid fatty acid
Solidification point	29.0°C.	——	
Melting point	33~35°C.	——	51~54°C.
Iodine value (Wijs)	191.5	240	12.7
Neutralization value	188.8	182.3	210.3
Mean molecular weight	297.0	307	266.8
Rhodan value		122	
Ether insoluble bromide		84.8%	

(II) Isolation of the Fatty Acids.

First of all, according to the Toyama's method, sodium salt of the highly unsaturated acid, which was soluble in acetone, was filtered from the insoluble salt of the mixed fatty acids; the insoluble part was dissolved by warming in a little quantity of 50% alcohol, and after neutralizing the excess of NaOH by acetic acid, the sodium salt was changed to the lead salt by pouring into the boiling 10% lead acetate; and was separated into two parts—the soluble and the insoluble in ether at ordinary temperature. Then, these parts were decomposed by 20% HCl and transferred as free fatty acids into the petroleum ether (b. p. $<50°$) and freeing from the solvent at low temperature under diminished pressure, each free fatty acid was prepared.

The quantities obtained from the mixed fatty acids were as follows:

(1) The highly unsaturated part, 36%, (2) The lowly unsaturated part, 33%, and (3) The saturated part, 31%. The degrees of the unsaturation and the average molecular weights, of the highly and the lowly unsaturated parts of the acids were as the following:

Table 3

	Highly unsaturated group	Lowly unsaturated group
Neutralization value	178.1	191.0
Mean molecular weight	315.2	293.8
Iodine value (Rosenmund und Kuhnheim's method)	356.0	155.5

(A) Researches of the Highly Unsaturated Part.

25 g. of the fatty acid were changed to its methyl ester under CO_2 atmosphere according to the Haller method, and it was fractionated by distilling in vacuo, and the results obtained were as given in Table 4.

Table 4

No. of fraction	Temp. of air bath	Distilling point		Yield	Iodine value	Ester value
		Temp. °C	Press. mm.			
1	190~200	120~144	0.4	2.2	169.6	200.8
2	200~215	160~173	1.0	4.2	272.4	184.5
3	220	185~193	1.5~2.0	2.8	339.0	176.4
4	227~230	197~205	2.0	6.0	362.1	168.5
5	235~240	205~210	2.0~2.5	7.0	274.0	164.7
Residue				small quantity		

In comparing the constants of each division in the foregoing table with the theoretical ones:—

	Iodine value	Ester value
$C_{18}H_{35-4}O_2$-CH_3	172.7	190.9
$C_{18}H_{35-6}O_2$-CH_3	260.8	192.2
$C_{20}H_{39-8}O_2$-CH_3	319.3	176.4
$C_{22}H_{43-10}O_2$-CH_3	369.0	163.1

It will be noted that about 60% of the highly unsaturated acids was clupanodonic acid $C_{22}H_{44-10}O_2$, and it is possible to infer that the rest was composed of the fatty acids, namely, $C_{20}H_{40-8}O_2$, $C_{18}H_{36-4}O_2$, ($C_{18}H_{36-4}O_2$?).

Furthermore, to ascertain these facts each division was changed to the reduced methyl ester ($C_nH_{2n-1}O_2-CH_3$), having them reduced in ether by hydrogen, using platinum black as the catalyser; the liberated acids ($C_nH_{2n}O_2$) were separated, the constants estimated, and the results obtained are as shown in Table 5.

Table 5

No. of fraction	Reduced methyl-esters				Freed reduced fatty acids			
	Saponification value		Melting point °C.		Saponification value		Melting point	
	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.	Exp.	Theor.
(1)	193.3	188.3 ($C_{18}H_{35}O_2-CH_3$)	26~27	38	195.2	197.3	58	69.3
(2)	180.3		36~37	46~47	178.0		67~68	
(3)	172.5	172.0 ($C_{20}H_{43}O_2-CH_3$)	41~42		173.5	179.8	69~70	75
(4)	167.0		45~46		167.5		74~75	
(5)	160.9	158.5 ($C_{22}H_{43}O_2-CH_3$)	47~48	53~54	194.0	165.0	79~80	79~80

To elucidate—it is inferrable that the division (1), the divisions (2) and (3), and the divisions (4) and (5) were composed of the unsaturated acids corresponding to stearic acid, arachidic acid, and behenic acid respectively.

(B) Researches of the Lowly Unsaturated Part.

(a) By the method of oxidation.

20 g. of the fatty acids were changed by Hazura's oxidizing method to oxidized acids using 3/4 % $KMnO_4$. These acids were extracted with petroleum ether and the unchanged fatty acids eliminated, and the quantity of the oxidized acids thus obtained was found to be about 10 g. This was again extracted for 20 hours with ether and the soluble part was separated from the insoluble.

(1) The soluble part in ether :– The quantity obtained was 4.5 g.; recrystallized twice from 90% alcohol and once from 95 % alcohol; mp. 118°~119°C.; no change of m. p. in recrystallizing from ethyl acetate; the crystal was flat irregular hexagonal; the neutralization value of the acids were 175.7 ~177.6 (The value as dihydroxy-stearic acid was 177.3).

(2) The insoluble part in ether :– Boiled 8 times in a liter of water for 1~3 hours each. The quantity of the soluble part in hot water was 1.7g., and that of the insoluble part 1.0 g. The former contracted at 120°C., and melted at 166~170°C., and a large part of it dissolved in 90 % alcohol. In crystallizing it formed flat hexagonal crystals; mp. 129~130°C.; the neu-

tralizing point 175.0. The latter is soluble in hot 90 % alcohol and when recrystallized from 95% alcohol, ethyl acetate, m.p. became definite at 119° C; the neutralization value was 174.6. Viz. even though the insoluble part had been extracted for 20 hours with ether, dihydroxy stearic acid still remaining was a large part of it, and tetra hydroxy and hexahydroxy stearic acids might have been present there but the quantities of these acids were so small that their further examinations could not be carried out.

(b) By the method of bromination.

4.2 g. of the fatty acids were treated by the usual method and the bromides were obtained.

(1) The insoluble bromides in ether :– The quantity obtained was 0.5 g.; the content of bromine in the insoluble part in hot benzene was 76.0 % and in the soluble part 71.0 % (The theoretical percentage as $C_{22}H_{34}O_2Br_{10}$ was 70.76%). Thus, it is readily seen that it was the decabromide of clupanodonic acid.

(2) The soluble bromides in ether :– Eliminating the excess of bromine and ether, and in extracting it with petroleum ether, a part became white precipitate. Besides, there was the coating of gelatinous substance on the vessel. The quantity obtained was small; the precipitate white, mp. 113°～115°C.; perhaps, this part might have been the tetrabromides of linolic acid and isolinolic acid.

(3) The soluble part in ether and petroleum ether :– The quantity obtained was 4.2 g. The bromine content was 39 %; the neutralization value was 120; (the theoretical value as the dibromide of oleic acid $C_{18}H_{38}O_2Br_2$–36.18 % Br; the neutralization value 126.9). It is clear that the greater part was the dibromide, though still mixed with tetrabromide.

From the foregoing results, it is seen that a large part of the division of this lowly unsaturated fatty acids was composed of oleic acid, small quantities of linolic acid and the highly unsaturated acids.

(c) Researches of the Saturated Fatty Acids.

20 g. of the fatty acids were made to be their methyl esters by the same method as that used for the division (A), and by distilling twice at the different temperatures under diminished pressure, the divisions obtained are as given in Table 6.

Table 6

The 1st distillation	The 2nd distillation						
	Temp. of air bath	Distilling point: Temp.	Distilling point: Press. mm.	Yield	Melting point°C.	Neutralization value	
I fraction	(1) 160	<140	2.0	0.4	16～19	228.5	Myristic acid theor. 231.8
	(2) 170	146～150	2.0	1.4	18～20	223.7	
0.8 mm.	(3) 173～190	150～160	2.6	1.0	19～22	216.6	
136～147°C.	(4)>190	160	3.0	1.7	26～27	206.7	

II fraction	(1) 175	130	1.0	1.3	23~24	208.7	Palmitic acid theor. 207.8
	(2) 180~190	143~148	1.0	4.0	23~24	204.0	
0.8 mm,	(3) 230	155	1.0	0.3	24	207.6	
150~155°C.	(4)	Residue		0.8		195.1	
III fraction	(1) 183	<140	2.0	0.6	24	202.3	
	(2) 190	145~150	2.0	0.5	24	205.3	
1.0 mm,	(3) 190~200	155~160	2.0	0.4	27~28	199.1	Stearic acid theor. 188.3
160~168°C.	(4) 205	165~168	2.0	1.2	27~32	194.2	
	(5)	Residue		1.7	31~33	186.1	
IV fraction				Small quantity			

From these divisions the liberated fatty acids were prepared, and the results determined of their melting points and neutralization values were as follows:

Table 7

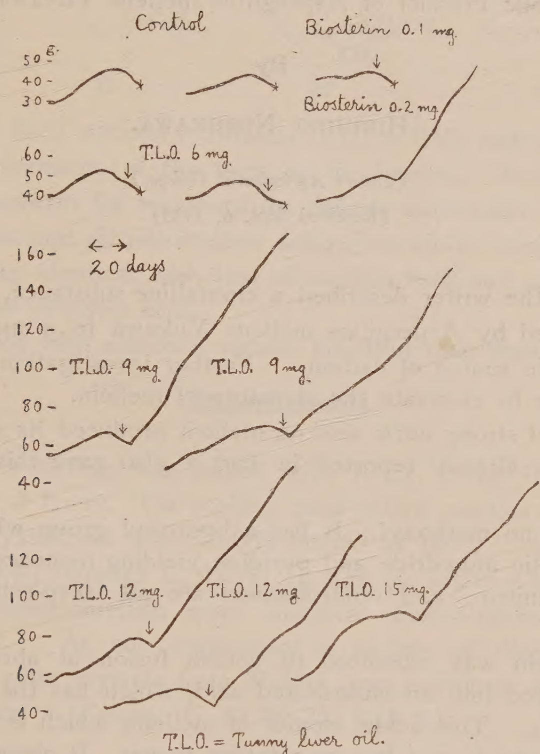
No. of fraction		Melting point °C.		Neutralization value	
		Exper.	theor.	Exper.	theor.
I	(1)	45~50		242.6	245.8 Myristic acid $C_{14}H_{28}O_2$
	(2)	47	53.7	235.5	
	(3)	61~62		220.8	
	(4)	58~59		217.5	
II	(1)	61~62	62.5	217.3	218.9 Palmitic acid $C_{16}H_{32}O_2$
	(2)	62~63		215.1	
	(3)	57~58		213.5	
	(4)	57~60		198.0	
III	(1)	—		—	
	(2)	55~56		211.5	
	(3)	55~56		206.5	
	(4)	68~69	72.0	199.0	197.3 Stearic acid $C_{18}H_{36}O_2$
	(5)	68~69		195.0	

(III) The Vitamin-A Potency of Tunny Liver Oil.

Albino rats weighing about 40 g. were fed with the vitamin-A free diet and, when their weight diminished and xerophtalmia appeared, the tunny liver oil mixed at the rate of 20 % with olive oil was given to them through the mouth. The sample oil was prepared in the manner as follows; the fresh liver of tunny caught in February was dehydrated by anhydrous Na_2SO_4 and was extracted with ether at low temperature. As shown in the diagram in case 0.2 mg. or more of the Biosterin was given to each of the animals per day, they were perfectly cured, while the tunny liver oil showed its complete curative power when 9 mg. or more of it was given.

Considering from the fact that relatively large amount of the Biosterin was required at the present test, it is inferrable that the curative power of the tunny liver oil is approximate to that of a commercial cod liver oil. This may also be verifi ed from the fact that its color reaction by antimony

trichloride is about the same as that of the Shimoda's cod liver oil on market.



Summary

(1) The isolation and determination of fatty acids of tunny liver oil were performed, and further the examination of the curative value of its vitamin-A was carried out.

(2) The fatty acids contents of the liver oil, collected in spring, were about as follows:

Oleic acid	30%	Clupanodonic acid	22%
Arachidonic acid	20%	Palmitic acid	19%
Stearic acid	7%	Myristic acid	5%

Small amount of Linolic acid.

(3) The curative power of the vitamin of this oil was determined by biological test, and 9 mg. of the oil per day for one albino rat was found to be sufficient.

Before closing this article, the writer wishes to express to Profs. U. Suzuki and M. Yamagawa his sincere acknowledgement and appreciation for their kind direction given him throughout this research work.

Biochemistry of Filamentous Fungi III.

A Metabolic Product of Aspergillus melleus Yukawa. Part II.

By

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(*Tottori Agricultural College.*)

(Received Sept. 6, 1933)

In Part I[1] the writer described a crystalline substance, mellein $C_{10}H_{10}O_3$, which is produced by Aspergillus melleus Yukawa in a medium containing sugar as the sole source of carbon. Further investigation is now reported which may serve to elucidate the structure of mellein.

Action of hot strong nitric acid on mellein produced its dinitro-derivative. Mononitromellein already reported in Part I also gave this substance when further nitrated.

Mellein has no methoxyl. It has a hydroxyl group which is acetylated by means of acetic anhydride and pyridine yielding monoacetylmellein. Monoacetyl-, mononitro-, and dinitromellein are all laevo-rotatory as mellein itself.

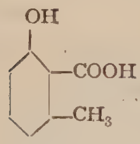
When mellein was submitted to potash fusion at about 200°, it was smoothly converted into an unsaturated acid, which has the same molecular formula, $C_{10}H_{10}O_3$. This acidic isomer of mellein, which is now named melleic acid, is monobasic and has no rotatory power. It absorbed catalytically two atoms of hydrogen giving a saturated acid, $C_{10}H_{12}O_3$.

Methylation of melleic acid with diazomethane gave monomethyl ester of melleic acid which gives a violet $FeCl_3$ reaction and is readily reconverted into melleic acid on treatment with alkali. Acetylation with acetic anhydride and pyridine produced its monoacetyl derivative which is a monobasic acid and gives no $FeCl_3$ reaction.

Potash fusion of mellein in the neighbourhood of 300° proceeded with vigorous effervescence and produced along with a trace of cresol a fair amount of an acid which, from analyses, melting points and other properties of itself and its acetyl derivative, is identical with 6–hydroxy–2–methylbenzoic acid (I) obtained by Anslow & Raistrick[2] as a metabolic product of Penicillium griseo-fulvum Dierckx. Melleic acid too was decomposed into this acid when fused with KOH.

From the facts mentioned above the formula of melleic acid will probably be represented by (II).

(1) Bull. Agric. Chem. Soc. Jap., **9**, 107 (1933).
(2) Anslow & Raistrick: Biochem. J., **26**, 43 (1931).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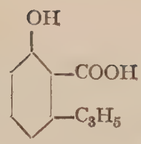
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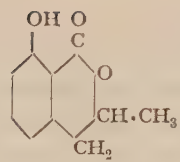
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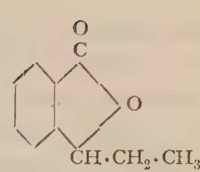

IV

To mellein itself structural formulae, (III) or (IV), may be assigned which satisfy the requirements of the facts so far known. Further experimental evidence will however be necessary for definite conclusion.

Both melleic and dihydromelleic acids give violet coloration with $FeCl_3$, colour tone being identical with that of mellein itself and again indistinguishable from that of salicylic acid.

Mellein gives with alcoholic potash beautiful lilac fluorescence.

Experimental.

Dinitromellein.

One gram of mellein was boiled with 10 c.c. conc. HNO_3 (sp. gr. 1.4) under reflux for 2 hours. On cooling, pale yellow needles separated (0.85 g), which after recrystallized from MeOH melted at 160°. (Found: C, 44.73; H, 3.37. $C_{10}H_8O_7N_2$ requires C, 44.78; H, 2.99 %. $[\alpha]_D^{32} = -508°.68$). Further nitration of mononitromellein gave identical dinitro-derivative, m.p. 160° alone or mixed. At one experiment a variety of dinitro compound of m.p. 125°, thin plate crystals from MeOH, was produced; it cannot however be prepared again. (Found: C, 44.38; H, 3.15 %).

Acetylmellein.

1 g of mellein was incubated with 4 c.c. acetic anhydride and 8 c.c. pyridine for 2 days at 35°. When the mixture was diluted with water and acidified with H_2SO_4, crystalline grains separated (0.8 g). Further 0.2 g was cropped from mother liquor by extraction with ether. Recryst. from water, thick hexagonal plates, m.p. 126°. (Found: C, 65.36; H, 5.55. $C_{12}H_{12}O_4$ requires C, 65.45; H, 5.45 %. $[\alpha]_D^{28} = -171°.80$).

Melleic acid.

1 g of mellein was fused with 10 g of KOH at 200°. The fusion proceeded without effervescence. The melt was dissolved in water and saturated with CO_2. Ether extracted practically nothing from it. On acidifying the solution with H_2SO_4 colourless needle crystals separated (0.8g), which melted at 170° after recrystallisation from water. Extraction of the filtrate with ether gave further 0.2 g of impure substance. (Found: C, 67.67; H, 5.78. $C_{10}H_{10}O_3$ requires C, 67.42; H, 5.62 %. Molecular weight estimated by titration. Found: 178.9. Calc.: 178). Melleic acid closely resembles salicylic acid in appearance.

Dihydromelleic acid.

0.5 g of melleic acid was catalytically hydrogenated in an ether solution, 0.2 g Pd–$BaSO_4$ being used as catalyst. The reaction finished in a few minutes. Residue from the ether (yield theoretical), when recrystallized from water, separated in colourless needles similar to melleic acid. M.p. 116°. (Found: C, 66.71; 6.78. $C_{10}H_{12}O_3$ requires C, 66.67; H, 6.67 %).

Melleic acid methyl ester.

An excess of diazomethane in ether solution was added to the solution of melleic acid in the same solvent. After immediate brisk evolution of gas ceased, ether driven off, the residue, for purification, was dissolved in alcohol, cooled in ice, and precipitated by adding ice-cold water. It has weak but characteristic smell. (Found: C, 68.72; H, 6.52. $C_{11}H_{12}O_3$ requires C, 68.75; H, 6.25 %). Dihydromelleic acid gave a liquid derivative when treated with diazomethane as above. Mellein did not react with diazomethane under the same experimental condition. Melleic acid was regenerated when the methyl ester was hydrolysed by warming with dilute NaOH and then acidified. The substance obtained melted at 170°, alone or mixed with an authentic specimen of melleic acid.

Acetylmelleic acid.

0.5 g of melleic acid was incubated at 35° with 2 c.c. acetic anhydride and 4 c.c. pyridine for 4 days, The mixture was cooled, diluted with water, acidified with H_2SO_4 and extracted with ether. The syrupy residue from the ether solidified to a crystalline magma while left in vacuum over KOH. Colourless angular grains, freed from mother liquor on porous porcelain, melted at 110°. No suitable solvent for recrystallization having been found, the substance was analysed without further purification. (Found: C, 65.02; H, 5.53. $C_{12}H_{12}O_4$ requires C, 65.45; H, 5.45 %). Titration with *N*/10 NaOH gave an equivalent of 223.3, assuming this to be a monobasic acid. Theoretical 220. An excess of *N*/10 NaOH was added to neutralised solution of the acid, the mixture boiled for 2 hours under reflux, cooled, and excess of alkali titrated with *N*/10 HCl. Acidity equivalent to monoacetyl was produced during hydrolysis.

6–Hydroxy–2–methylbenzoic acid.

1 g of mellein was submitted to potash fusion with 10 g of KOH at 300∼310°. Vigorous evolution of gas took place. The fused mass was dissolved in water, saturated with CO_2, and extracted with ether. When the ether was driven off, a minute quantity of oily matter remained having strong smell of cresol. The solution after being acidified was again extracted with ether. The residue from the ether (0.7 g) crystallized from water in colourless needles, m.p. 170°. (Found: C, 62.90; H, 5.45. $C_8H_8O_3$ requires C, 63.16; H, 5.26 %. Mol. wt, by titration 150. Calc. 152). The substance

described by Anslow & Raistrick has m.p. 170~171°. Other properties are also identical.

Acetyl derivative of 6-hydroxy-2-methylbenzoic acid.

0.4 g of 6-hydroxy-2-methylbenzoic acid, 2 c.c. acetic anhydride and 4 c.c. pyridine were mixed and incubated at 30° for 3 days. The mixture was diluted with H_2O, acidified with H_2SO_4, and extracted with ether. The residue from the ether crystallized from benzene in colourless prisms (lst crop 0.2 g), m.p. 131°. (Found: C, 61.89; H, 5.29. $C_{10}H_{10}O_4$ requires C, 61.85; H, 5.15%). Titration with N/10 NaOH to phenolphthalein gave an equivalent of 194.6. Theoretical 194. Boiling with an excess of N/10 NaOH and back titration with N/10 HCl showed that acidity equivalent to monoacetyl was produced during the hydrolysis. Acetyl derivative prepared by Anslow & Raistrick melts at 131°.

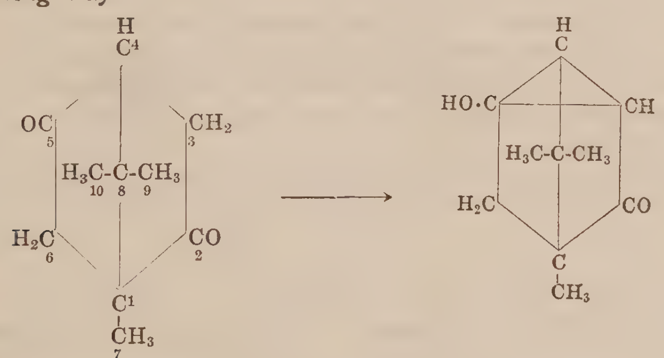
On the Physiologically Active Isomer of Bredt's 5-Oxo-camphor.

By

Kunijiro TAKEUCHI and Yoshikazu SAHASHI.

(Received August 29, 1933)

During the studies on the camphor group the present authors have observed that the Bredt's 5-oxo-camphor, prepared from borneol[1] can be transformed into its so-called enol-isomer by prolonged boiling with hexane in the following way:



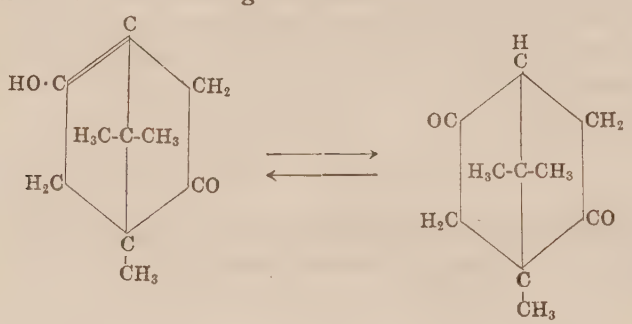
Bredt's 5-oxo-camphor (prismatic crystal)   Enol-isomer (irregular plate)

The prismatic crystals are converted thereby into irregular plates and at the same time acquire the property of stimulating the heart of animals

while the keto-isomer has no such activity.

This observation led the authors to examine the so-called allo-*p*-oxo-camphor prepared by Tamura, Asahina and co-workers,[2] to determine whether or not the active component in the said preparation is identical with the enol-isomer mentioned above. For this purpose, 5-oxy-camphor was first prepared from the urine of dogs administered with camphor per os and it was converted into allo-*p*-oxo-camphor by carefully oxidizing with sulphuric acid and sodium bichromate, following the method of above authors.

This preparation is stated by these authors to be the mixture of keto- and enol-isomers of the following formulae:



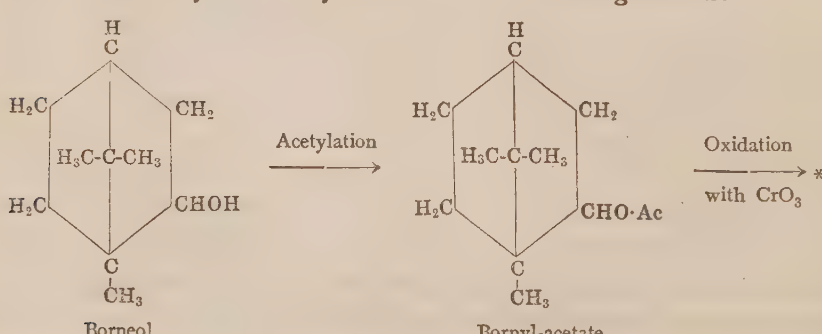
(Allo-*p*-oxo-camphor)

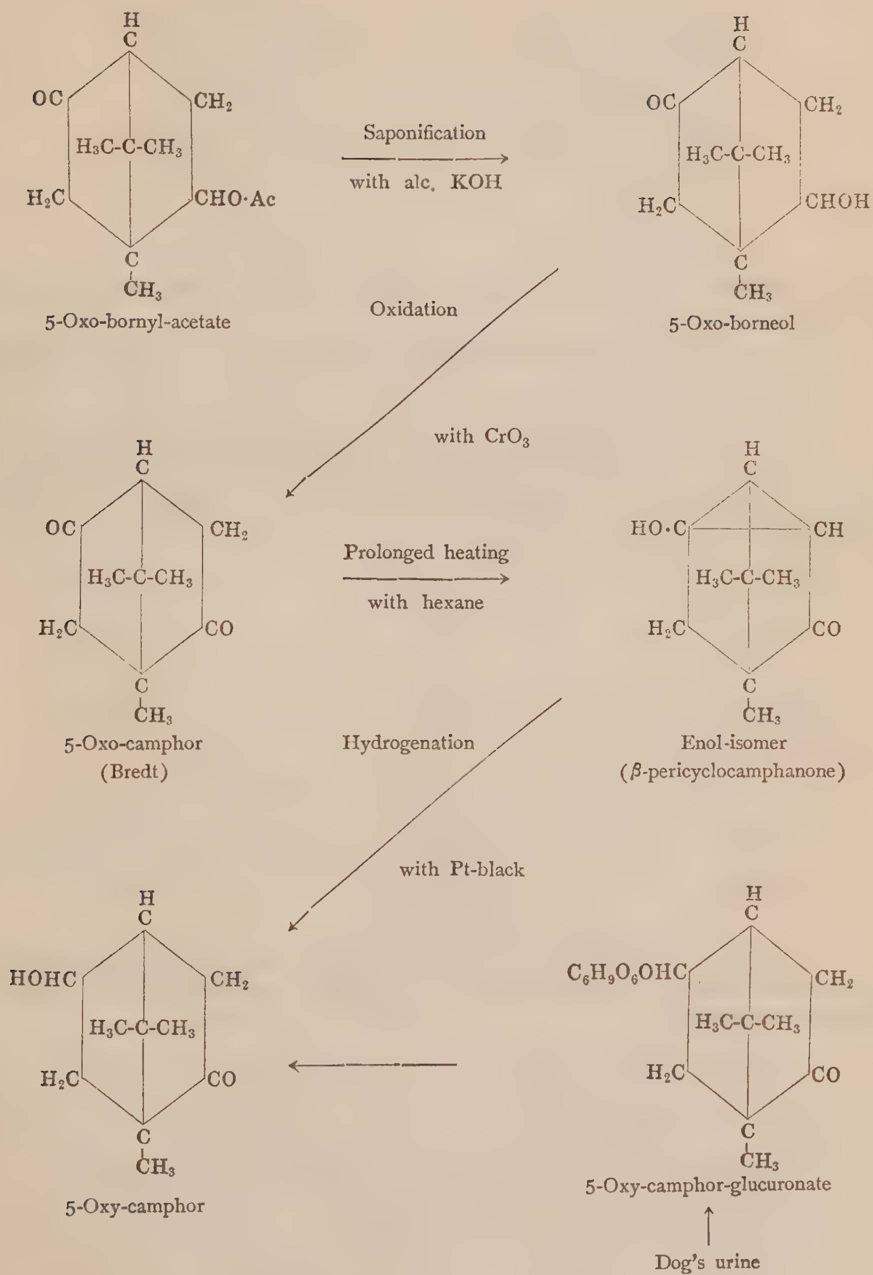
Under the microscope, it was revealed to be the mixture of two kinds of crystals, i. e. the prisms and irregular plates as stated by these authors.

The present authors have now observed that by prolonged boiling with hexane the prismatic crystals in the above preparation were gradually converted into plate ones and the latter was exactly identical, both in its chemical properties as well as in its physiological behaviours, with the enol-isomer of the synthetic 5-oxo-camphor, the stimulating action upon the heart of animals being estimated to be quite similar in both cases.[3]

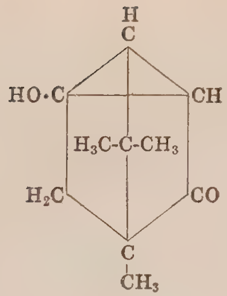
Further, the present authors have regenerated 5-oxy-camphor by reducing the enol-isomer of the synthetic 5-oxo-camphor and proved it to be identical with the preparation, isolated from dog's urine according to the method of Asahina and co-workers.

This relation may be briefly stated in the following scheme:

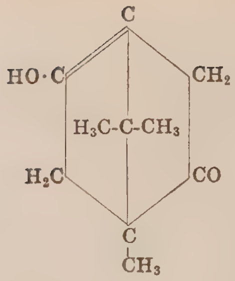




The question, whether the enol-isomer mentioned above is represented by the formula (I) or (II) remains to be settled.



(I)

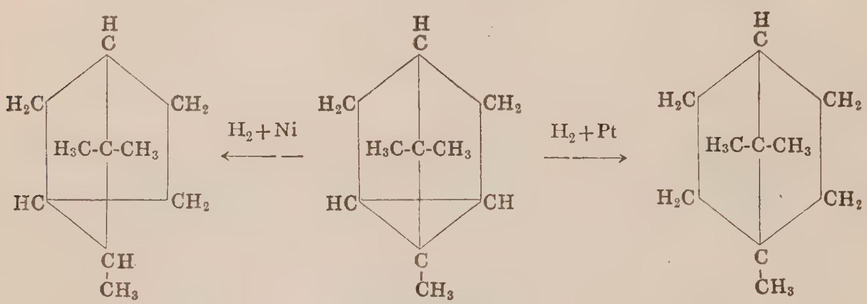


(II)

The present authors are however inclined to accept the formula (I) from the following reasons:

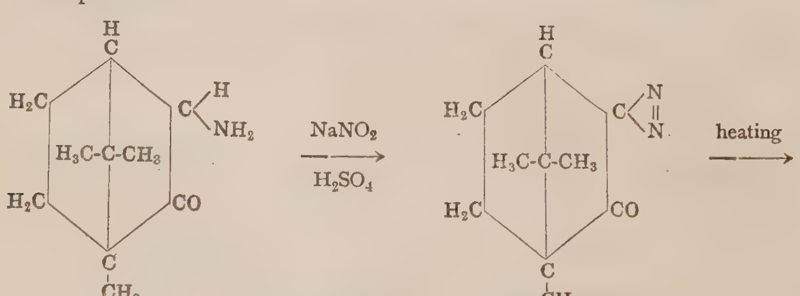
(1) There is no absorption spectra or any chemical reaction indicating the presence of double bond.

(2) 5-Oxo-camphor of keto form (prismatic crystals) cannot be hydrogenated with hydrogen and platinum while the enol-isomer is slowly reduced to 5-oxy-camphor.[4] This is in analogy with the fact that α-pericyclocamphane can be reduced to camphane by hydrogen and platinum and to iso-camphane by hydrogen and nickel.

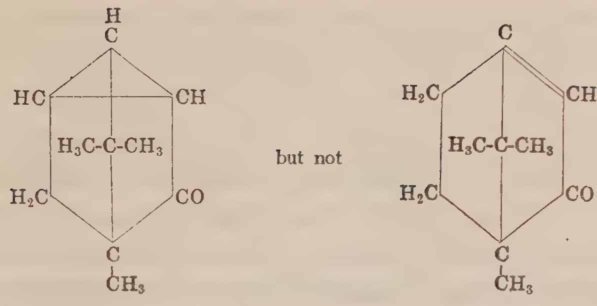


Iso-camphane α-Pericyclo-camphane (α-Tricyclene) Camphane

(3) Bredt and Holz[5] proved experimentally that diazocamphor derived from α-amino-camphor does not form camphenone by heating but gives β-pericyclocamphanone.

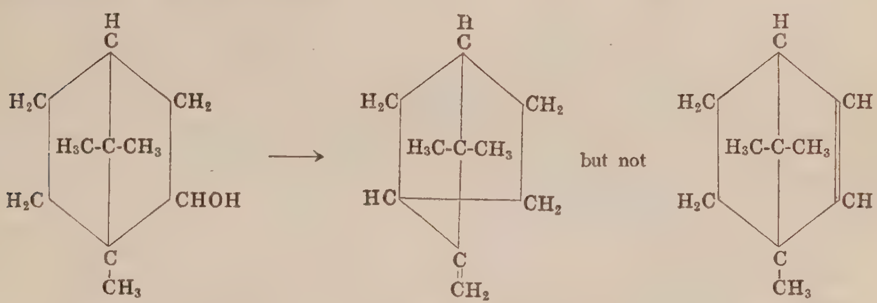


α-Aminocamphor Diazocamphor



β-Pericyclo-camphanone Camphenone

Further it is well known that borneol is transformed to camphene by dehydrating agents but not to bornylene.



Borneol Camphene Bornylene

So the existence of double bond in the enol-isomer of 5-oxo-camphor is very improbable and the authors believe that the formula (I) (tricyclene form) is in better accordance with various facts.

Experimental

I. *Synthetical Preparation of 5-Oxo-camphor from Borneol after Bredt.*

(1) 5-Oxo-borneol: Bornyl-acetate [b p/4 mm$=80\sim81°$ (uncorr.)] was first prepared from borneol by heating with acetic anhydride and it was oxidized into 5-oxo-bornyl-acetate [b p/$4\sim5$ mm $= 125\sim127°$ (uncorr.)] with chromic anhydride in glacial acetic acid. The latter was then saponified with alcoholic potash in the usual way and after evaporating off the alcohol, the residual solution was saturated with CO_2 and extracted with ether. The 5-oxo-borneol thus obtained was recrystallized from alcohol; m p $= 232\sim235°$ (uncorr.), $[\alpha]_D^{18.0°} = +82.1°$ in alcohol ($c = 2.46$), (Bredt's 5-oxo-borneol; m p $= 238\sim246°$ (uncorr.), $[\alpha]_D^{14.5°} = +71°$).

The authors are indebted to Messers T. Iki and K. Tanaka for analyses.

Analysis: 3.781 mg subs. gave 9.919 mg CO_2, 3.274 mg H_2O;
$C = 71.55\%$, $H = 9.69\%$.
4.021 " " " 10.564 " ", 3.509 mg H_2O;
$C = 71.65\%$, $H = 9.76\%$.
Calc. for $C_{10}H_{16}O_2$ $C = 71.37\%$, $H = 9.59\%$.

(2) 5-Oxo-camphor: This was obtained by oxidizing the 5-oxo-borneol, mentioned above, with chromic acid in boiling water-bath and was recrystallized from ligroin in prismatic crystals (Photo. 5); m p = 207°(uncorr.), $[\alpha]_D^{17.0°} = +103.5°$ in ligroin ($c = 2.025$). (Bredt's 5-oxo-camphor; m p = 206.5 ~206.7°, $[\alpha]_D^{13.5°} = +103°$).

Analysis: 3.882 mg subs. gave 10.282 mg CO_2, 2.950 mg H_2O;
$C = 72.23\%$, $H = 8.50\%$.
4.111 " " " 10.870 " ", 3.137 mg H_2O;
$C = 72.11\%$, $H = 8.54\%$.
Calc. for $C_{10}H_{14}O_2$ $C = 72.28\%$, $H = 8.44\%$.

(3) The enol-isomer of 5-oxo-camphor: When 5-oxo-camphor (prismatic crystals) obtained as above was boiled with ligroin or hexane for a long time,* it was converted into its enol-isomer, which crystallized in colourless irregular plates (Photo. 6).

The preparation treated with ligroin: m p = 209~210° (uncorr.); $[\alpha]_D^{17.0°} = +144.6°$ in ligroin ($c = 2.109$).

The preparation treated with hexane: $[\alpha]_D^{17.0°} = +147.3°$ in ligroin ($c = 1.988$).

The preparation purified from ligroin, was analyzed.

Analysis: 4.040 mg subs. gave 10.688 mg CO_2, 3.150 mg H_2O;
0.1993 g subs. 26.92 g benzol gave $\Delta = 0.22$;
$C = 72.15\%$, $H = 8.72\%$, mol. wt. = 168.
Calc. for $C_{10}H_{14}O_2$ $C = 72.28\%$, $H = 8.44\%$, mol. wt. = 166.

(4) Semicarbazone of the enol-isomer: When the enol-isomer, mentioned above, was treated in the usual way with semicarbazide hydrochloride and Na-acetate and the reaction product was recrystallized from alcohol, the mono-semicarbazone was obtained as colourless needles; soluble in alcohol, m p = 230~231° (uncorr.).

Analysis: 3.810 mg subs. gave 0.6070 c.c. N_2 (758.5 mm, 19°C);
$N = 18.60\%$.
3.445 " " " 0.5468 " N_2 (762.5 mm, 18.5°C);
$N = 18.56\%$.
Calc. for $C_{11}H_{17}O_2N_3$ $N = 18.80\%$.

* We have also prepared the above enol-isomer from Bredt's prismatic 5-oxo-camphor by heating at 200°C in a sealed tube, which was previously evacuated by means of high vacuum pump.

It was observed that the above enol-isomer had the activity upon the heart of animals similar to the preparation obtained from dog's urine.

II. *Regeneration of 5-Oxy-camphor by the Reduction of the Enol-isomer of 5-Oxo-camphor.*

(1) Five grams of enol-isomer of 5-oxo-camphor, synthetically prepared from borneol, were dissolved in 5 g acetic acid and treated with hydrogen gas for 50∼100 hours, using platinum-black as catalyst. The solution was then diluted with water, filtered from the catalyzer, neutralized with sodium carbonate and extracted with ether. The etherial solution was evaporated to dryness and the crystalline residue thus obtained was treated with 10 % caustic potash and converted into acetate. By saponifying the latter, 5-oxy-camphor was regenerated. When recrystallized from large amount of ligroin it formed nice characteristic crystals (Photo. 7); m p = 220∼222° (uncorr.), $[\alpha]_D^{18.0°} = +18.5 \sim 50.2°$ in ligroin, $[\alpha]_D^{18.5°} = +47.5°$ in alcohol ($c = 2.862$). Mixed with pure specimen of 5-oxy-camphor (m p = 222.5°, $[\alpha]_D^{18.5°} = +47.6°$) prepared from dog's urine, no depression of melting point was observed.

Analysis: 4.340 mg subs. gave 11.295 mg CO_2, 3.679 mg H_2O;
C = 70.98%, H = 9.48%.
4,025 〃 〃 〃 10.510 〃 〃, 3.430 mg H_2O;
C = 71.21%, H = 9.53%.
Calc. for $C_{10}H_{16}O_2$ C = 71.37%, H = 9.59%.

(2) 5-Oxy-camphor-semicarbazone was prepared from the above sample in the usual way; m p = 220° (uncorr.).

Analysis: 3.960 mg subs. gave 0.5998 c.c N_2 (770 mm, 17.5°C);
N = 18.03%.
Calc. for $C_{11}H_{19}O_2N_3$ N = 18.6%.

(3) 5-Acetoxy-camphor was prepared from the same sample by heating with acetic anhydride in the usual method and purified by distilling in vacuum; colourless liquid, b p (25 mm) = 137∼140°C (uncorr.).

Analysis: 5.054 mg subs. gave 12.730 mg CO_2, 3.982 mg H_2O;
C = 68.69%, H = 8.81%.
5.018 〃 〃 〃 12.633 〃 〃, 3.910 mg H_2O;
C = 68.66%, H = 8.71%.
Calc. for $C_{12}H_{18}O_3$ C = 68.57%, H = 8.5%.

(4) 5-Acetoxy-camphor-semicarbazone was prepared from the above acetate; m p = 197∼200°C (uncorr.).

Analysis: 3.476 mg subs. (dried at 100°C) gave 7.338 mg CO_2, 2.578 mg H_2O;
$C = 57.57\%$, $H = 8.28\%$.
3.648 〃 〃 (dried at 150°C) 〃 7.780 〃 〃, 2.660 mg H_2O;
$C = 58.17\%$, $H = 8.15\%$.
Calc. for $C_{13}H_{21}O_2N_3$ $C = 58.42\%$, $H = 7.90\%$.
3.040 mg subs. (dried at 150°C) gave 0.4185 c.c. N_2 (763 mm, 28°C);
$N = 15.63\%$.
2.877 〃 〃 (dried at 150°C) 〃 0.4057 c.c. N_2 (759 mm, 28°C);
$N = 15.97\%$.
Calc. for $C_{13}H_{21}O_2N_3$ $N = 15.73\%$.

III. *Isolation of 5-Oxy-camphor from Dog's Urine.*

(1) 5-Oxy-camphor was isolated from dog's urine according to the method of Y. Asahina and M. Ishidate, and after boiling with 10 % caustic potash for an hour, it was converted into the acetate. The latter was then saponified with alcoholic potash in the usual way and recrystallized from ligroin (Photo. 10):

m p = 222.5°C (uncorr.); $[\alpha]_D^{24.0°} = +43.9°$ in abs. alcohol ($c = 2.956$) or
$[\alpha]_D^{18.5°} = +47.1°$ in abs. alcohol ($c = 2.564$).

Analysis: 3.792 mg subs. gave 9.842 mg CO_2, 3.330 mg H_2O;
$C = 70.79\%$, $H = 9.82\%$.
4.500 〃 〃 〃 11.700 〃 〃, 3.878 mg H_2O;
$C = 70.91\%$, $H = 9.64\%$.
Calc. for $C_{10}H_{16}O_2$ $C = 71.37\%$, $H = 9.59\%$.

(2) 5-Acetoxy-camphor was prepared from the above sample; b p (25 mm) = 150～151°C (uncorr.).

Analysis: 4.444 mg subs. gave 11.141 mg CO_2, 3.418 mg H_2O;
$C = 68.37\%$, $H = 8.60\%$.
Calc. for $C_{12}H_{18}O_3$ $C = 68.57\%$, $H = 8.5\%$.

(3) 5-Acetoxy-camphor-semicarbazone; m p = 180～190°C (uncorr.).

Analysis: 4.210 mg subs. gave 8.964 mg CO_2, 3.047 mg H_2O;
$C = 58.07\%$, $H = 8.09\%$.
4.088 〃 〃 〃 9.5468 c.c. N_2 (764 mm, 22.5°C);
$N = 15.53\%$.
Calc. for $C_{13}H_{21}O_2N_3$ $C = 58.42\%$, $H = 7.90\%$,
$N = 15.73\%$.

The above data agree fairly well with those reported by Y. Asahina and M. Ishidate* as shown in the following Table:

---

* We have also isolated 5-oxy-camphor form the urine of rabbits administered with camphor per os, and confirmed it to be identical with the preparation obtained from dog's urine.
m p = 215～217°C (uucorr.).
Analysis: 3.325 mg subs. gave 8.660 mg CO_2, 2.880 mg H_2O;
$C = 71.04\%$, $H = 9.69\%$.
3.483 〃 〃 〃 9.088 〃 〃 3.024 mg H_2O;
$C = 71.16\%$, $H = 9.71\%$.
Calc. for $C_{10}H_{16}O_2$ $C = 71.37\%$, $H = 9.5\%$.

	Present authors	Y. Asahina and M. Ishidate
5-Oxy-camphor	m p = 222.5°C; $[\alpha]_D^{24.0°} = +43.9°$ in alcohol; C = 70.85%, H = 9.73%.	m p = 222°C; $[\alpha]_D^{30.0°} = +43.2°$ in alcohol; C = 70.9%, H = 9.7%.
5-Acetoxy-camphor	b p (25 mm) = 150∼151°C; C = 68.37%, H = 8.60%.	b p (27 mm) = 158∼160°C.
5-Acetoxy-camphor-semicarbazone	m p = 180∼190°C; C = 58.07%, H = 7.90%. N = 15.53%.	m p = 180∼185°C; N = 16.01%.

IV. *Oxidation of 5-Oxy-camphor.*

5-Oxo-camphor: The purified 5-oxy-camphor mentioned above was converted into 5-oxo-camphor by oxidizing with sulphuric acid and potassium bichromate at 50∼60° in the same way as reported by Asahina and Ishidate and recrystallized from hexane; m p = 197∼200° (uncorr.), $[\alpha]_D^{23.0°} = +70.8°$ in abs. alcohol ($c = 2.988$).

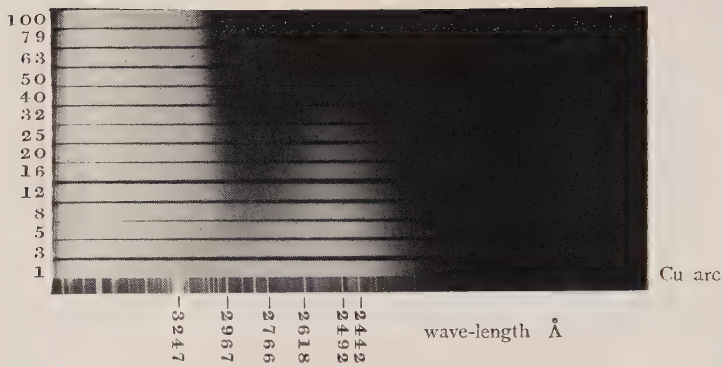
Analysis:	3.288 mg subs. gave 8.640 mg CO_2,	2.770 mg H_2O;
		C = 71.66%, H = 9.42%.
	Calc. for $C_{10}H_{14}O_3$	C = 72.28%, H = 8.44%.

The 5-oxo-camphor thus obtained was revealed to be the mixture of two kinds of crystals i. e. the prisms and irregular plates (Photo. 8) but on prolonged boiling with hexane, the prismatic crystals were gradually converted into plate form (Photo. 9); m p = 195∼200°C (uncorr.), $[\alpha]_D^{23.0°} = +101.6°$ in alcohol ($c = 3.266$).

Analysis:	3.683 mg subs. gave 9.635 mg CO_2,	3.030 mg H_2O;
		C = 71.35%, H = 9.20%.
	Calc. for $C_{10}H_{14}O_2$	C = 72.28%, H = 8·44%.

The stimulant action of the above compounds upon the heart of animals were determined according to Langendorff's method, and the results are shown in the following Photos (Photo. 11∼20).

The authors wish to express their sincere thanks to Professors U. Suzuki, T. Shimamura and T. Yabuta for their kind advices throughout this work. Thanks are also due to Dr. S. Kato and Mr. T. Shimamoto for their kind help.



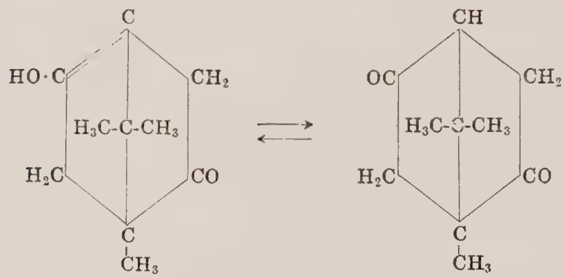
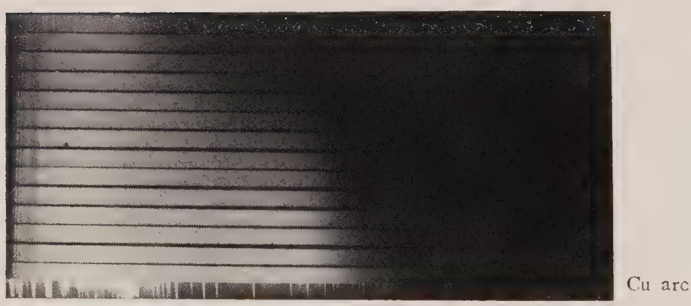


Photo. 1—Commercial Vitacamphor
(0.0825 g in 50 c.c. water).



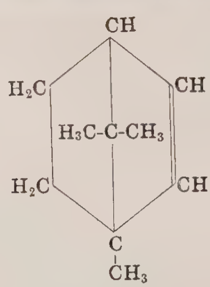
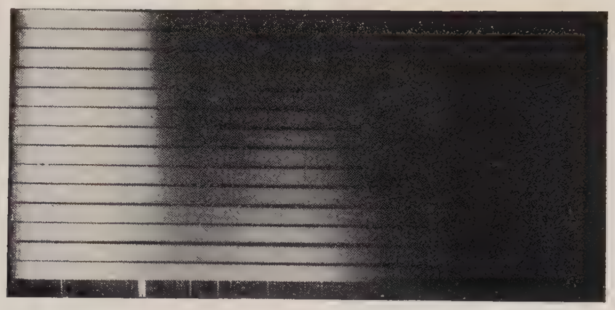


Photo. 2—Bornylene (0.0094 g in 50 c.c. ether).



Cu arc

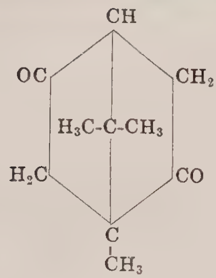
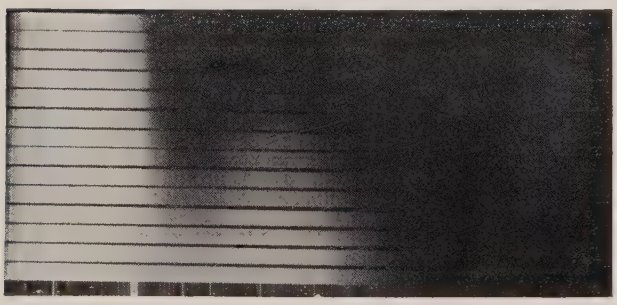


Photo. 3—Bredt's 5-oxo camphor (prismatic form)
(0.0830 g in 50 c.c. ether).



Cu arc

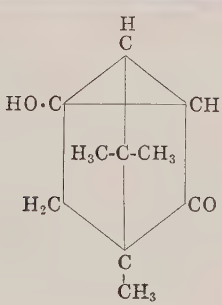


Photo. 4—Synthetic enol-form of 5-oxo-camphor
(plate form)
(1/100 mol ether)

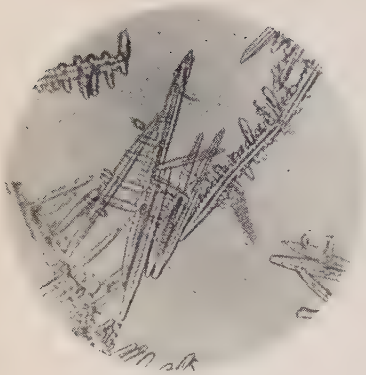


Photo. 5—Bredt's 5-oxo-camphor (prismatic form)

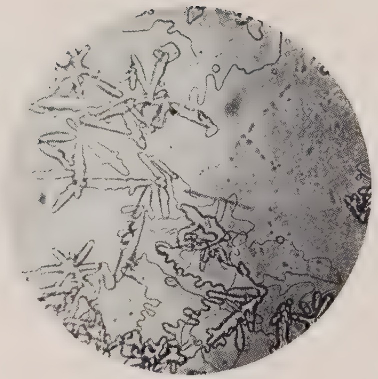


Photo. 8—5-Oxo-camphor obtained from 5-oxy-camphor in dog's urine (the mixture of two kinds of crystals).

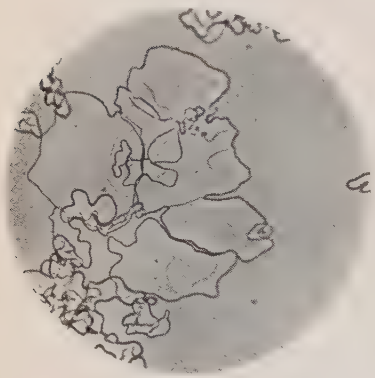


Photo. 6—Enol-isomer of Bredt's 5-oxo camphor (plate form).

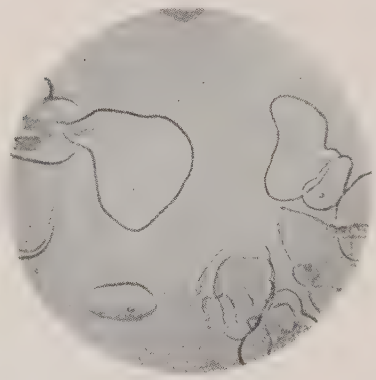
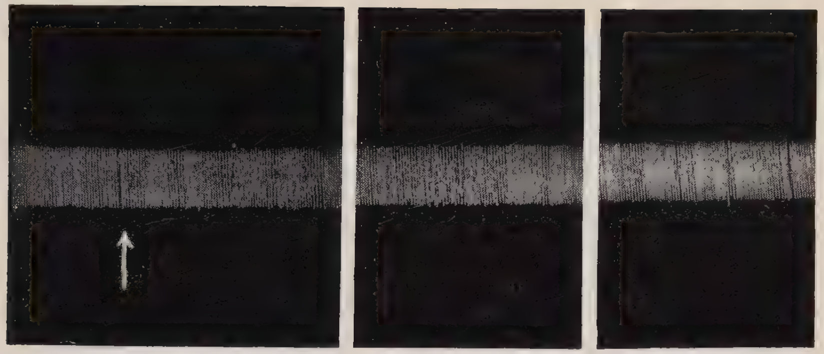


Photo. 9—Enol-isomer of 5-oxo-camphor from 5-oxo-camphor in pog's urine.

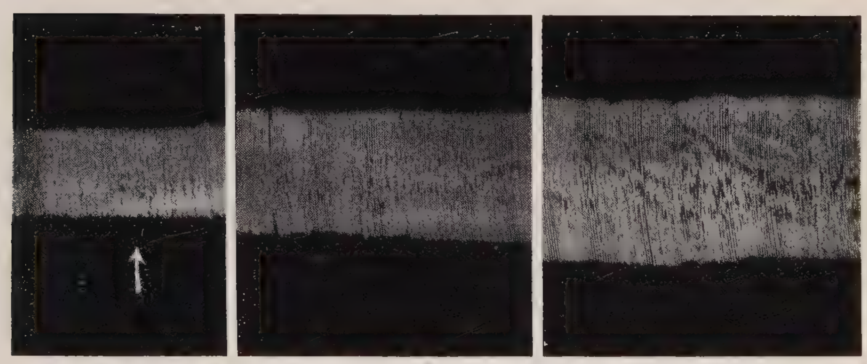
Photo. 7—Synthetic 5-oxy-camphor.

Photo. 10—5-Oxy-camphor obtained from dog's urine.



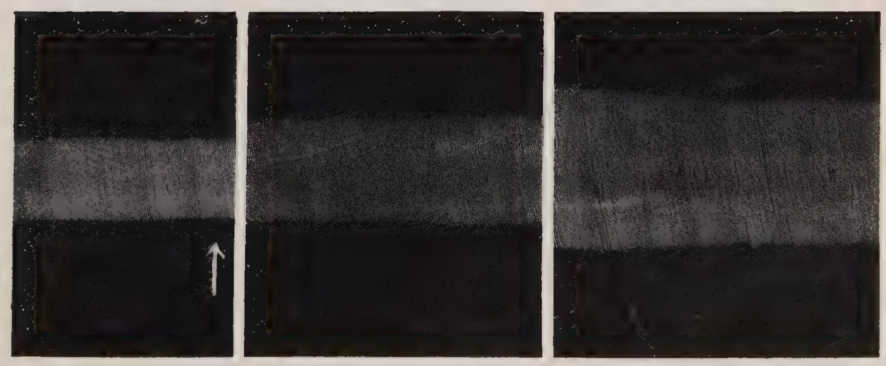
after 5 min after 22 min

Photo. 11—Showing the action of synthetic 5-oxo-camphor (m p 207°, $[\alpha]_D^{17°} = +103$) 0.1%, 0.5 c.c, upon the heart of guinea pig.



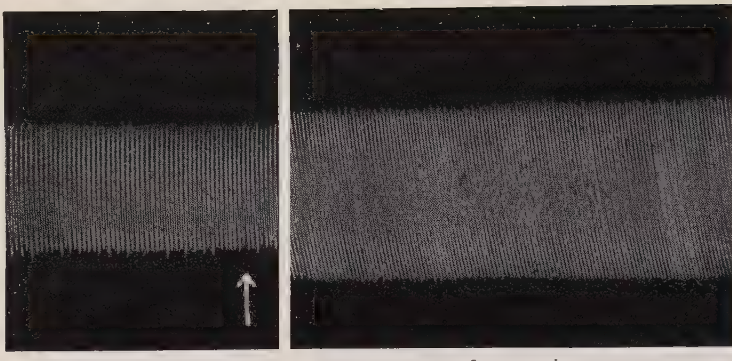
after 8 min after 20 min

photo. 12—Stimulant action of synthetic 5-oxo-camphor (plate form; m p 210°, $[\alpha]_D = +144.6°$) upon the heart of guinea pig; 0.1%, 0.5 c.c.



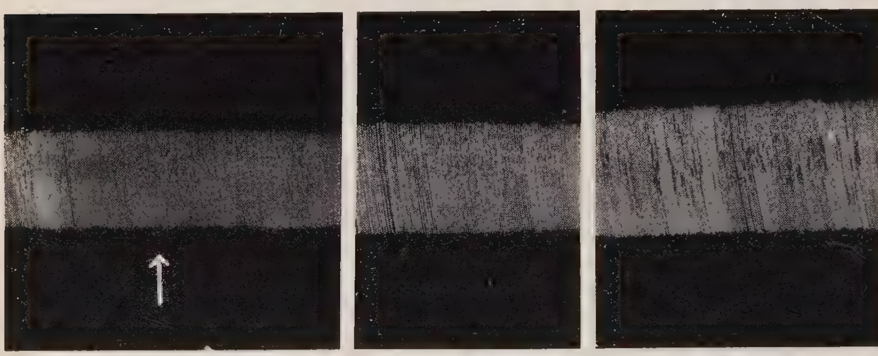
after 10 min after 18 min

Photo. 13—Stimulant action of synthetic 5-oxo-camphor (plate-form; m p 210°, $[\alpha]_D = +144.6°$) upon the heart of guinea pig; 0.5%, 0.2 c.c.



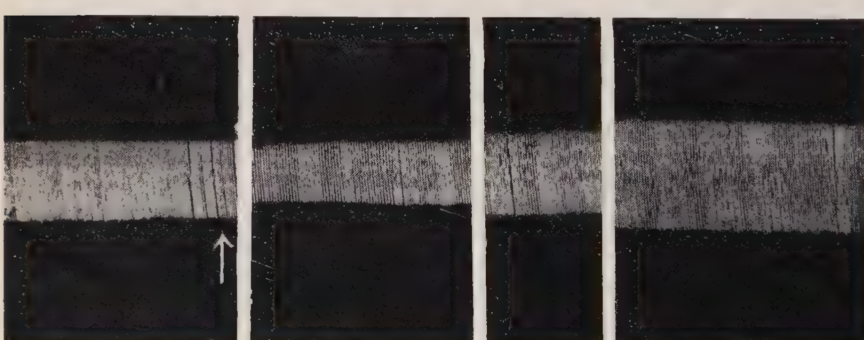
after 18 min

Photo. 14—Stimulant action of synthetic 5-oxo-camphor (plate-form; m p 210°, $[\alpha]_D=+144.6°$) upon the heart of rabbit; 0.5%, 0.4 c.c.



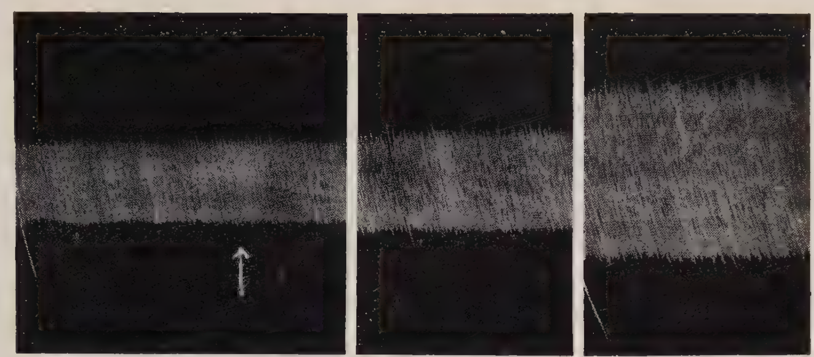
after 5 min after 12 min

Photo. 15—Stimulant action of synthetic 5-oxy-camphor (m p 220~221°, $[\alpha]_D=+18°$) upon the heart of guinea pig; 0.5 %, 0.2 c.c.



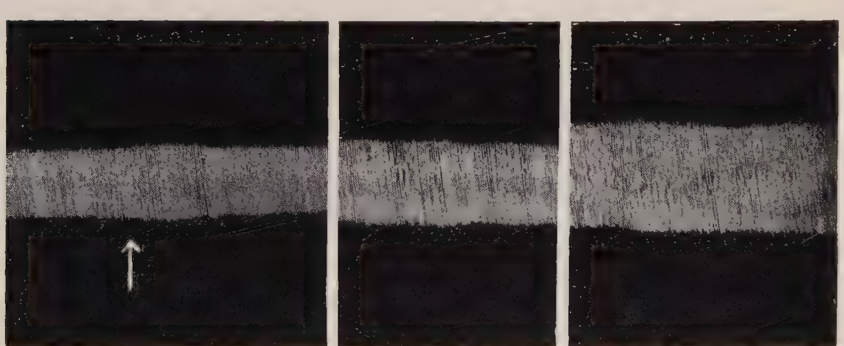
after 8 min after 40 min

Photo. 16—Stimulant action of synthetic 5-oxy-camphor (m p 220~221°), $[\alpha]_D=+18°$) upon the heart of guinea pig; 0.5 %, 0.5 c.c.



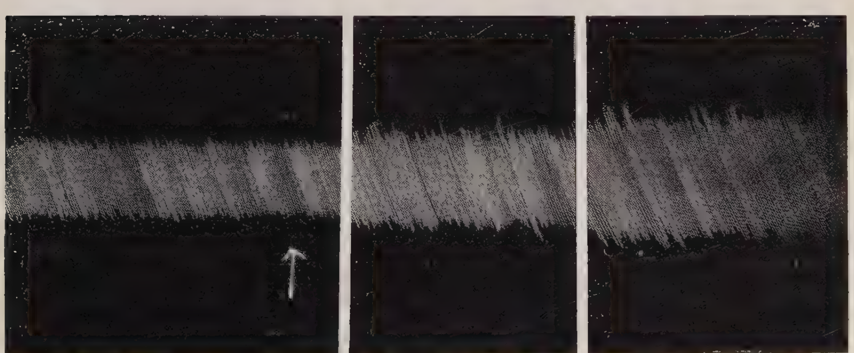
after 8 min after 20 min

Photo. 17—Stimulant action of 5-oxy-camphor from dog's urine (m p 222.5°, $[\alpha]_D = +43.9$) upon the heart of rat; 0.5%, 0.5 c.c. aq.



after 12 min after 30 min

Photo. 18—Stimulant action of 5-oxo-camphor from dog's urine (mixture of plate and prisms; m p 195～200°C, $[\alpha]_D = +70°$) upon the heart of guinea pig; 0.5%, 0.2 c.c.



after 7 min after 17 min

Photo. 19—Stimulant action of 5-oxo-camphor from dog's urine (plate form; m p 195～200°C, $[\alpha]_D = +101.6°$) upon the heart of rat; 0.5%, 0.5 c.c.

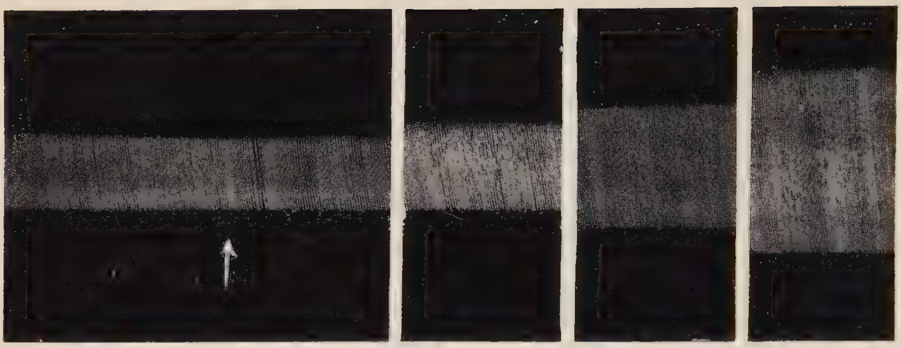


Photo. 20—Stimulant action of 5-oxo-camphor from dog's urine (plate form; m p = 195~200°, $[\alpha]_D = +101.6°$) upon the heart of guinea pig; 0.5%, 0.2 c.c.

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On the Organic Bases, especially Agmatine of "Di-Saké".

By

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(Recieved July 6, 1933)

Recently one of the authors (Bull. Agr. Chem. Soc. Japan, Vol. 8, Nos. 10~12, 151) reported that the occurrence of hypoxanthine, arginine, choline and ammonia in "di-saké", which is a kind of "saké" resulting after pressing the fermented rice.

In this experiment, the authors isolated hypoxanthine, choline, ammonia, betaine and agmatine from "di-saké".

Experimental part.

Experiment 1.

For the isolation of the organic bases 20 litres of the "di-saké" were evaporated under reduced pressure to a small volume, almost equal to 1/3 of the original. After this operation the protein substance and other impurities were removed by lead acetate, and excess of the lead by H_2SO_4, and then the organic bases were precipitated by phosphotungstic acid. And according to the general method the precipitate formed by phosphotungstic acid was fractionated into three fractions, and in each fraction researches were made about the organic bases.

(1) *Purine base-fraction* (*hypoxanthine*)

The yield of the base from this fraction was 0.60 g. as hydrochloride. On analysing this hydrochloride, the following result was obtained:

0.1196 g. subst.	0.03483 g. N	29.12% N
Calc. for $C_5H_4N_4O \cdot HCl \cdot H_2O$ (Hypoxanthine hydrochloride)		29.41% N

The chloroaurate of the base formed yellow prisms, decomposed at 248°C.

0.1106 g. subst.	0.0453 g. Au	40.96% Au
0.0871 g. subst.	0.0358 g. Au	41.10% Au
Calc. for $C_5H_4N_4O \cdot HCl \cdot AuCl_3$ (Hypoxanthine chloroaurate)		41.42% Au

(2) *Arginine-fraction* (*agmatine*)

The yield of the base from this fraction was 1.40 g. as nitrate.

The nitrate of the base, easily soluble in water and given strong Sakaguchi's reaction, crystallized in bright colourless thin leaflets, and melted at 150~151°C.

Its analytical results agreed with the nitrate of the compound, which has the formula $C_5H_{14}N_4$.

No.	Subst. mg.	CO_2 mg.	H_2O mg.	C%	H%	N%
(1)	3.233	2.840	1.878	23.96	6.49	——
(2)	2.963	2.600	1.720	23.93	6.49	——
(3)	3.486	3.048	2.040	23.90	6.53	——
(4)	5.217	1.4504 c.c. N (23.0°C., 763.0 m.m.)				32.19
(5)	3.307	0.9261 c.c. N (23.5°C., 763.5 m.m.)				32.39
Calc. for $C_5H_{14}N_4 \cdot 2HNO_3$				23.42	6.30	32.81

The nitrate was converted into picrate by adding Na-picrate to its aqueous solution. The picrate formed deep yellow prisms, hardly soluble in water, and decomposed at 239°C.

0.0412 g. subst.	0.0755 g. nitronpicrate	77.55% picric acid
Calc. for $C_5H_{14}N_4 \cdot 2C_6H_3N_3O_7$ (Agmatine picrate)		77.88% picric acid

The chloroaurate of the base was prepared from the hydrochloride, which was obtained by decomposing the picrate by HCl.

0.2387 g. subst.	0.1166 g. Au	48.85% Au
0.3251 g. subst.	0.1593 g. Au	49.00% Au
Calc. for $C_5H_{14}N_4 \cdot 2HCl \cdot 2AuCl_3$ (Agmatine chloroaurate)		48.67% Au

The chloroplatinate of the base, easily soluble in water, crystallized in orange yellow prisms and decomposed at 216°C.

0.1152 g. subst.	0.0420 g. Pt	36.46% Pt
Calc. for $C_5H_{14}N_4 \cdot 2HCl \cdot PtCl_4$ (Agmatinechloroplatinate)		36.14% Pt

From these results, the organic base obtained from the arginine-fraction of "di-saké" is no doubt agmatine.

(3) *Lysine-fraction (betaine and choline)*

The hydrochloride obtained by this fraction, was treated with absolute alcohol and separated into two portions.

(a) Insoluble portion by absolute alcohol: Yield, 0.80 g. It was colourless short prisms, and the derivatives were prepared as follows:

The picrate formed greenish yellow prisms and melted at 181°C

The chloroaurate of the base, hardly soluble in water, crystallized in golden yellow plates of pearly lustre and decomposed at 242°C.

0.2279 g. subst.	0.0983 g. Au	43.13% Au
0.1973 g. subst.	0.0849 g. Au	43.03% Au
Calc. for $C_5H_{11}NO_2 \cdot HCl \cdot AuCl_3$ (Betainechloroaurate)		43.14% Au

The chloroplatinate, easily soluble in water, formed orange yellow prisms and melted at 246°C.

0.2322 g. subst.	0.0699 g. Pt	30.10% Pt
Calc. for $(C_5H_{11}NO_2 \cdot HCl)_2PtCl_4$ (Betainechloroplatinate)		30.25% Pt

(b) Dissolved portion by absolute alcohol: Saturated alcoholic solution of $HgCl_2$ was added to this portion.

The hydrochloride of the base obtained from the $HgCl_2$-precipitate, formed colourless, hygroscopic, and large prisms and gave the alloxan reaction. Yield: 4.20 g. as hydrochloride. The chloroaurate formed yellow mossy crystals, and was sparingly soluble in water; the melting point was determined as 259°C.

0.1671 g. subst.	0.0739 g. Au	44.23% Au
0.1716 g. subst.	0.0757 g. Au	44.11% Au
0.1628 g. subst.	0.0724 g. Au	44.47% Au
Calc. for $C_5H_{14}NOCl \cdot AuCl_3$ (Cholinechloroaurate)		44.49% Au

Experiment II.

In the second experiment on the isolation or organic bases from "di-saké", 26 litres of the sample was employed and treated like the first experiment; and the quantities of substances isolated by the second experiment were as follows

Hypoxanthine (as hydrochloride)	0.70 g.
Agmatine (as picrate)	4.60 g.
Betaine (as hydrochloride)	0.40 g.
Choline (as chloroaurate)	32.00 g.

Summary.

(1) In the above experimental result the nitrogenous compounds isolated from "di-saké" are:

	In the first experiment (Sample 20 litres)	In the second experiment (Sample 26 litres)
Hypoxanthine (as hydrochloride)	0.60 g.	0.70 g.
Agmatine	1.40 g. (as nitrate)	4.60 g. (as picrate)
Betaine (as hydrochloride)	0.80 g.	0.40 g.
Choline	4.20 g. (as hydrochloride)	32.00 g. (as chloroaurate)
Ammonia	4.73 g. (determined)	——

(2) It is the interesting fact that ths "di-saké" contains fairly amount of agmatine, which had never been isolated from any other fermentation products.

Feeding Experiments with Decomposition Products of Proteins. III.

By

Shiro MAYEDA

(Received June 12, 1933.)

In the previous communications,[1] the author has shown, from the results of feeding experiments with white rats, that the proteins in diet can be entirely replaced by the biuret-free acid-hydrolytic products of proteins when supplemented with tryptophane.

Continuing the studies on this subject, the author has carried out further experiments with the mixture of purified amino acids of the following composition: glycocoll 2%, *dl*-alanine 13%, *l*-leucine 15%, *l*-proline 4%, *l*-oxyproline 4%, *l*-tyrosine 3%, *l*-phenylalanine 3%, *l*-cystine 3%, *d*-glutamic acid 15%, *dl*-aspartic acid 6%, *dl*-tryptophane 2%, mixture of *l*-histidine-, *d*-arginine- and *l*-lysine hydrochloride 30%. For the preparation of the diet used in the experiment, 15 parts of the above amino acid mixture were

added to 65 p. of starch, 15 p. of butter, 5 p. of McCollum's salt mixture, besides 5 p, of alcoholic extract of yeast. When rats were fed on this diet, they soon lost the appetite and the body weights rapidly decreased until all of them succumbed within 2～3 weeks, So it is evident that the above amino acid mixture could not substitute the proteins in diet, thus confirming the observations of Abderhalden,[(2)] U. Suzuki,[(3)] Mitchell,[(4)] McClendon[(5)] and Rose.[(6)(7)]

When, however, the mono-amino fraction of the acid hydrolysate i.e. the filtrate of the phosphotungstic precipitate, was given together with tryptophane and diamino acids, the animals could grow normally, indicating that a certain indispensable factor for growth, which is just lacking in the above amino acid mixture is present in the mono-amino fraction.

W. C. Rose,[(7)] who is working on the same line, has also come to the same conclusion that the substance essential for growth is contained in the butyl alcohol-soluble portion i.e. in the mono-amino fraction of casein hydrolysate.

With the purpose of isolating this substance, the present author has now separated the mono-amino fraction of the acid hydrolysate of fish meat proteins into 4 parts i. e. (1) fraction less soluble in water, (2) mono-amino dicarboxylic fraction, (3) fraction soluble in alcohol, (4) mono-amino mono-carboxylic fraction. Each fraction was then tested for its supplementing effect by adding it, to the extent of 1.5%, to the basal diet containing the above amino acid mixture. In this way the last (4) fraction alone i. e. the mono-amino mono-carboxylic fraction was proved to be effective for the growth of animals, so it was further converted into copper salts[(8)] by boiling with copper carbonate and again separated into 3 parts by treating with water and methyl alcohol successively i. e. (1) the part insoluble in water, (2) insoluble in methyl alcohol, (3) soluble in methyl alcohol. Each fraction was then treated with hydrogen sulphide to remove the copper and tested on rats. The result has shown that the favorable growth is only induced by the addition of the 3rd fraction i. e. the copper salt, soluble in methyl alcohol.

In the next experiment, the mono-amino mono-carboxylic fraction i. e. the 4th fraction of the above mentioned was converted into zinc salt[(9)] by boiling with zinc carbonate and separated into 3 parts i. e. (1) insoluble in water, (2) insoluble in ethyl alcohol and (3) soluble in ethyl alcohol. Each fraction was freed from zinc by treating with hydrogen sulphide and evaporated to dryness. Feeding experiments with these preparations have proved that the 3rd fraction i. e. the zinc salt, soluble in ethyl alcohol alone is effective.

Taking these facts in consideration, the author has proceeded as follows. The mono-amino mono-carboxylic fraction obtained from the acid hydrolysate of fish meat protein was converted into zinc salts and, after complete drying,

it was pulverized and extracted several times with boiling absolute alcohol. The alcoholic solution was then evaporated in vacuum to dryness and extracted again with cold absolute alcohol. This operation was repeated until there was no insoluble residue left. The zinc salt obtained by evaporating the alcohol was then dissolved in water, decomposed with hydrogen sulphide and converted into copper salt in the usual way. The copper salt thus obtained was now treated with cold methyl alcohol, the methyl alcoholic solution was evaporated, dissolved in water and treated with hydrogen sulphide to remove the copper, and filtered from the copper sulphide. When the filtrate thus obtained was evaporated at a lower temperature, a colorless crystalline substance separated out which was recrystallized from 80% alcohol.

Feeding experiments were carried out by adding these crystals to the basal diet containing the amino acid mixture above mentioned. When the rats were previously fed on the basal diet alone they rapidly lost their body weight, but when supplied with 0.5% of the above crystals they began to recover in weight, though slowly. When the amount was doubled, the growth was more rapid, thus in one experiment the rats gained in weight from 74 and 75 grs. up to 88 and 97 grs., resp. in 26 days. So it is clear that this substance played a remarkable rôle upon the nutrition of rats.

This substance crystallizes in colorless needles. Heated in a capillary, it softens at 208–9°, and melts at about 220° with decomposition. It gives no biuret reaction, but gives a typical bluish violet coloration with ninhydrin. It is also precipitated by mercuric acetate in alkaline medium like many amino acids.

The results of analysis are as follows:

4.433 mg. Subst. 7.365 mg. CO_2 and 3.380 mg. H_2O
4.157 mg. Subst. 0.3900 cc. N (756.5 mm., 25.5°C)

	C	H	N
Found :	45.31%	8.53%	10.41%
Calc. for $C_5H_{11}O_3N$: (oxy-amino-valerianic acid)	45.11%	8.27%	10.53%

Apparently it agrees with the formula of oxy-amino-valerianic acid, but it is not yet sure whether this substance is really a single one, and further investigations are necessary to decide whether or not it is identical with oxyvaline, obtained by Schryver and Buston[9] from oat protein.

The author expresses his sincere thanks to Prof. U. Suzuki for his kind advice and encouragement throughout the work.

(Prof. U. Suzuki's Laboratory, The Institute for Physical and Chemical Research.)

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Untersuchungen über die Enzyme von Bombyx mori, L.

III. Mitteilung. Über die Tyrosinase und Katalase des Blutes der Seidenraupen.

Von

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(Eingegangen am 24, Juli 1933.)

Eingehende Angaben über die Tyrosinase und Katalase bei Insekten sind sehr selten. In Fortsetzung der Untersuchungen über die Enzyme bei den Seidenraupen,[1][2] teilt nun der Verfasser die Ergebnisse seine Forschungen über diese beiden Enzyme des Blutes derselben mit.

1. Über die Tyrosinase.

(A) *Einrichtung der Versuche*:— Die für meine Versuche verwendeten Seidenraupen wurden im Frühling 1933 unter gleichen Bedingungen auf ge zogen. Die quantitative Bestimmung der Tyrosinase geschah der zahlreichen, vergleichenden Untersuchungen wegen durch die einfache, etwas modifizierte BACHsche Methode. Zu diesem Zweck wurden 15 ccm 0.03 proz. 1-Tyrosinlösung, 3 ccm M/3 Phosphatpufferlösung von pH 6.64 (mit Ausnahme von Versuch 1.), 1.5 ccm Wasser und 0.5 ccm mit 0.85 proz. NaCl-Lösung dreifach verdünntes Blut vermischt und 30 Minuten lang bei 30° (mit Ausnahme von Versuch 2.) gehalten. Dann wurde die Reaktion durch 2 ccm 10 proz. Schwefelsäure sistiert and mit 0.01 N $KMnO_4$-Lösung bis zur Entfärbung titriert.

(B) *Optimale pH*:– Versuch 1. China-7-B, 2. Tag im V. Lebensalter der Raupe.

pH	6.47	6.64	6.81	7.17	7.38	7.73	8.04
0.01 N $KMnO_4$. ccm	3.8	4.4	4.0	3.8	3.4	3.2	2.8

(C) *Optimaltemperatur*:– Versuch 2. China-7-B, 4. Tag im V. Lebensalter der Raupe.

Temp.°	23	30	37	45	55	65
0.01 N $KMnO_4$. ccm	1.2	4.1	5.4	4.8	2.8	0.6

(D) *Geschlechtsunterschied*:– Versuch 3. China-7-D, 4. Tag im V. Lebensalter der Raupen.

	♀				♂			
	Gruppe 1	Gruppe 2	Gruppe 3	Mittel	Gruppe 1	Gruppe 2	Gruppe 3	Mittel
0.01 N $KMnO_4$. ccm	2.8	2.8	3.2	2.9	4.4	3.8	4.0	4.1

(E) *Unterchied zwischen gut gewachsenen und schlecht gewachsenen Raupen*:– Versuch 4. Japan-110-E, 4. Tag im V. Lebensalter.

	Gut gewachsene Raupen.				Schlecht gewachsene Raupen.			
	Gruppe 1	Gruppe 2	Gruppe 3	Mittel	Gruppe 1	Gruppe 2	Gruppe 3	Mittel
0.01 N $KMnO_4$. ccm	3.8	3.8	4.4	4.0	3.8	4.4	3.6	3.9

(F) *Aenderungen durch Hungerzustand bei den Raupen*:– Versuch 5. Europa-7-A, 4. Tag im V. Lebensalter.

Hungerstunden	1	6	24	50
0.01 N $KMnO_4$. ccm	1.2	2.4	1.6	0.8

(G) *Unterschied zwischen den verschiedenen Rassen der Seidenraupen*:– Versuch 6. V. Lebensalter. 0.01 N $KMnO_4$ ccm.

Rasse / Tage	Japan-110-E	Japan-110-G	China-7-B	China-7-D	Europa-7-A	Europa-7-C
2	6.4	6.0	—	3.4	2.8	0.8
4	4.0	3.4	6.2	3.2	1.2	0.8
7	4.4	4.0	10.6	4.8	2.2	2.4

(H) *Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori, L.*:- Versuch 7. Europa-7-A.

	Raupe	Einspinnen des Kokons		Puppe		
Tage	Reife	2	4	1	3	5
0.01 N $KMnO_4$ ccm	4.2	0.6	0.2	0.4	0.6	0.4

	Puppe			Schmetterling	
Tage	8	11	13	1	3
0.01 N $KMnO_4$ ccm	0.3	0.4	0.4	5.6	6.2

II. Über die Katalase.

(A) *Einrichtung der Versuche*:— Als Substrat benutzte ich der gleichzeitig durchgeführten, zahlreichen Versuche und ferner der kräftigen Katalasewirkung des Bluts wegen etwas konzentrierte, nämlich ca. 0.3 Proz. H_2O_2-Lösung. 25 ccm H_2O_2-Lösung, 3 ccm M/3 Phosphatpufferlösung von pH 6.64 (mit Ausnahme von Versuch 1.), 1.5 ccm Wasser und 0.5 ccm mit 0.85 proz. NaCl-Lösung dreifach verdünntes Blut wurden gemischt und nach 30 Minuten bei 30° (mit Ausnahme von Versuch 2.) in Kölbchen mit 10 proz. Schwefelsäure eingelassen. Dann wurde die nicht zersetzte Menge Wasserstoffsuperoxyd duch Titration mit einer 0.01 N $KMnO_4$-Lösung bestimmt.

(B) *Optimale pH*:— Versuch 1. China-7-B, 3. Tag im V. Lebensalter der Raupe.

pH	6.47	6.64	6.81	7.17	7.38	7.73	8.04
Gespaltene H_2O_2 mg	18.70	20.94	20.20	19.45	17.95	16.64	14.59
Spaltung %	24.21	26.81	26.14	25.18	23.74	21.30	18.88

(C) *Optimaltemperatur*:— Versuch 2. China-7-B, 4. Tag im V. Lebensalter der Raupe.

Temp °	5	15	23	30	37	45
Gespaltene H_2O_2 mg	11.05	14.28	15.13	11.56	8.84	7.17
Spaltung %	14.30	18.49	19.59	14.96	11.44	9.24

(D) *Geschlechtsunterschied*:— Versuch 3. China-7-D, 4. Tag im V. Lebensalter der Raupen.

	♀				♂			
	Gruppe 1	Gruppe 2	Gruppe 3	Mittel	Gruppe 1	Gruppe 2	Gruppe 3	Mittel
Gespaltene H_2O_2 mg	14.21	13.09	13.46	13.59	17.95	17.58	18.70	18.08
Spaltung %	18.40	16.82	17.43	17.55	23.74	22.75	24.21	23.57

(E) *Unterschied zwischen gut gewachsenen und schlecht gewachsenen Raupen*:— Versuch 4. China-7-D, 7. Tag im V. Lebensalter.

	Gut gewachsene Raupen				Schlecht gewachsene Raupen			
	Gruppe 1	Gruppe 2	Gruppe 3	Mittel	Gruppe 1	Gruppe 2	Gruppe 3	Mittel
Gespaltene H_2O_2 mg	16.08	15.71	15.33	15.77	13.46	12.72	12.34	12.84
Spaltung %	20.82	20.33	19.75	20.30	17.43	16.46	15.97	16.62

(F) *Änderungen durch Hungerzustand bei den Raupen*:— Versuch 5. Europa-7-C, 4. Tag im V. Lebensalter.

Hungerstunden	1	6	24	50
Gespaltene H_2O_2 mg	7.48	7.85	6.73	8.23
Spaltung %	9.68	10.17	8.71	10.65

(G) *Unterschied zwischen den verschiedenen Rassen der Seidenraupen*:— Versuch 6. V. Lebensalter.

Tage	2		4		7	
Rasse	Gespaltene H_2O_2 mg	Spaltung %	Gespaltene H_2O_2 mg	Spaltung %	Gespaltene H_2O_2 mg	Spaltung %
Japan-110-E	16.08	20.82	14.96	19.30	15.71	20.33
Japan-110-G	24.31	31.47	20.19	26.14	19.82	25.66
China-7-B	——	——	10.71	13.86	15.47	20.03
China-7-D	19.89	25.75	13.47	17.44	14.59	18.89
Europa-7-A	8.16	10.56	6.68	8.65	7.85	10.16
Europa-7-C	6.29	8.14	7.48	9.68	10.10	13.07

(H) *Veränderungen im Laufe von drei Entwicklungsperioden von Bombyx mori, L.*:— Versuch 7. Europa-7-A.

	Raupe	Einspinnen des Kokons		Puppe		
Tage	Reife	2	4	1	3	5
Gespaltene H_2O_2 mg	11.22	21.32	20.94	19.82	40.02	31.56
Spaltung %	14.52	27.60	27.09	25.66	51.81	40.85

	Puppe			Schmetterling	
Tage	8	11	13	1	3
Gespaltene H_2O_2 mg	35.36	14.11	11.97	18.50	25.57
Spaltung %	45.77	18.27	15.50	23.95	33.10

Zusammenfassung.

(1) Die Tyrosinase des Bluts von Bombyx mori wirkt am besten bei pH 6.6. Die Optimaltemperatur liegt bei 37°.

(2) Die Tyrosinasewirkung des Bluts ist bei den Männchen etwas stärker als bei den Weibchen, aber es besteht kein Unterschied zwischen den gut gewachsenen und den schlecht gewachsenen Raupen. Durch Hunger wird die Wirkung der Tyrosinase erst etwas stärker und dann allmählich schwächer.

(3) Der Tyrosinasegehalt des Bluts der japanischen und der chinesischen Rassen ist recht viel höher als derjenige der europäischen. Bei den reifen Larven ist die Tyrosinase sehr aktiv, aber mit dem Beginn des Spinnens der Kokons tritt eine rapide Abnahme auf und während der Puppenperioden bleibt sie bedeutend zurück. Dann wieder vermehrt sich die Tyrosinase in den Tagen des Schmetterlingslebens rasch bis zu einem Maximum.

(4) Die Blutkatalase von Bombyx mori zeigt die optimale Aktivität bei pH 6.6; ihr Temperaturoptimum liegt bei 23°.

(5) Die Katalasewirkung des Bluts ist bei den Männchen etwas stärker als bei den Weibchen und auch bei gut gewachsenen Raupen etwas höher als bei schlecht gewachsenen. Es wurde aber innerhalb der zwei Hungertage keine Änderung beobachtet.

(6) Der Katalasegehalt des Bluts der japanischen und der chinesischen Rassen ist recht viel höher als derjenige der europäischen. Beim Beginn des Kokonspinnens der reifen Larve findet eine rapide Zunahme der Katalaseaktivität statt, die ihr Maximum meist am dritten Tage der Puppenperiode erreicht. Dann sinkt die Katalase allmählich und wird in der Periode des Schmetterlingslebens wieder bedeutend stärker.

Literatur.

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On the Two Kinds of Saponin of Soya Bean.

By

Koji OKANO and Iwao OHARA.

(Received Oct. 21, 1933)

We have isolated easily and in large quantity the two kinds of saponin, crystal and amorphous, from the alcoholic extraction of soya bean as the by-product in the process of the alcoholic extraction method of the soya bean oil.[1] The crystalline saponin had been investigated by Y. Sumiki[2] already and he reported that it formed squama, melting at 222～4°, the molecular formula was $C_{49\sim52}H_{70\sim84}O_{21}$, by the hydrolysis sapogenin ($C_{32\sim35}H_{48\sim56}O_3$), glucose, rhamnose, arabinose and other unknow acidic substance were obtained, and its haemolytic and toxic power were very weak. E. Walz[3] had reported that there were three kinds of saponin in soya bean, but those details remained unexplained. We have compared the two saponins and further investigated them closely as the first step toward the utilization of alcoholic extract.

Experimental.

1) Isolation :— Alcoholic extract of soya bean, from which the bean oil was separated and alcohol was evaporated, is mixed with NaCl-solution and from separated precipitate the ether soluble matter is removed, then the residue is recrystallized from 80% alcohol repeatedly and crystal and amorphous ones are separated by fractional crystallization.

2) Molecular formulas and properties :—

(A) Crystalline saponin. mel. pt. 225～227°. Its properties are identical with those of Sumiki and natrium-salt is hexagonal plate, decomposing at 259°, both have not haemolytic power.

analysis ; $C_{48\sim50}H_{77\sim81}O_{18}$, cal. H 8.25～8.43%, C 61.21～61.92%
obs. H% 8.66 8.58 9.00 9.43, C% 61.03 61.04 61.07 61.86
mol. wt. cal. 941～969, obs. 960 964 992

(B) Amorphous saponin. mel. pt. 216～218°. White powder is recry-

stallized from butyl alcohol and its natrium-salt decomposes at 260° and both have not haemolytic power.

analysis ; $C_{45\sim51}H_{79\sim83}O_{19}$, cal. H 8.20~8.37%, C 60.56~61.26%.
obs. H% 8.86 8.83 8.67 8.52, C% 61.24 60.75 60.41 60.70.
mol. wt. cal. 971~999, obs. 1160 1154.

3) The products by the hydrolysis :—

(A) Hydrolysis. Each saponin is heated in 80% alcohol-5% sulfuric acid 5~40 hours and after evaporation of alcohol sapogenin insoluble in water is filtered off and from the water solution sulfuric acid is removed by neutralization with baryta and then bariumsalt of glucuronic acid is obtained by adding alcohol to the concentrated water solution and next from alcohol solution the mixture of monosaccharides is gained as syrupy mass.

(B) Glucuronic acid is so accertained that above obtained bariumsalt is estimated barium content (26.25%) and it forms the *p*-Br-phenyl-osazon-glucuronic acid-barium (mel. pt. 214°) and phenyl-osazon (mel. pt. 204°).

(C) Galactose from the fraction of monosaccharides is established by the formation of music acid (mel. pt. 216°) and methyl-phenyl-hydrazon (mel. pt. 191°). (after treating with diphenylhydrazin to remove the arabinose wich is derived from glucuronic acid).

(D) Rhamnose is proved as *p*-Br-phenylhydrazon (mel. pt. 213°) (after galactose above mentioned is removed) and phenylosazon (mel. pt. 161°). Above three products are identical in both crystal and amorphous saponin.

(E) Sapogenin is obtained five isomers as the period of hydrolysis and studied its mutual relation.

[1] shows the sapogenin from the crystalline saponin.
[2] 〃 〃 〃 〃 〃 amorphous saponin.

(A) Sapogenins soluble in carbon tetrachloride and ligroin.

(1) Yellow amorphous (hydralysis 5 hrs.)

[1] mel. pt. 75~80~128° [α]=+25.4°
[2] mel. pt. 95~120° [α]=+48.0°

↓ (hydrolysis in 80%. alcohol-5% H_2SO_4. 10~20 hrs.

(2) Needle crystal (〃)

[1] mel. pt. 235°. [α]=+74.0°
[2] mel. pt. 224~226 [α]=+87.3°

(hydrolysis above the same ↓ ↑ (1% alcoholic KOH 4 hrs. boiling

(3) Needle crystal (some what short) (hydrolysis 15~40 hrs.)

[1] mel. pt. 239° [α]=+81.7°
[2] mel. pt. 232° [α]=+107.8°

kalifusion

hydrolysis

(B) Sapogenins insoluble in the same solvent.

(1) Needle crystal (when ever) (soluble in chloroform)

[1] mel. pt. 260° [α]=0
[2] mel. pt. 293~294° [α]=+9.6°

↑ (1% alcoholic KOH 4 hrs. boiling

(2) Rectangle plate crystal (hydrolysis 40 hrs.) (insoluble in chloroform)

[1] mel. pt. 248~249° [α]=0
[2] mel. pt. 313° [α]=+115.8°

The products of kalifusion and hydrolysis of the (B) sapogenins are almost analogous to yellow amorphous and crystal of (A) sapogenins respectively, but those specific rotation deviates a little from these value.

These all sapogenins are recrystallized from methylalcohol or benzol, and soluble in alcohol, ether, but insoluble in water. They are not lacton and are neutral with the exception of faintly acid of (A) [1] which gives by the titration with alkali the twice of the value of molecular weight as monobasic acid. Moreover it is interesting that if these saponins and sapogenins are heated with copper oxide or lead chromate by means of Dumas method, they liberate the considerable volume of methan or ethan gas.

The analytical data of these sapogenins differ a little each other, therefore they are not true isomer, but we have been able to consider the following formula.

1. $C_{30\sim32}H_{48\sim52}O_3$

		H%	C%	mol. wt.
cal.		10.53~10.83,	78.95~79.34,	456~484.
obs.	(A) [1]	10.51 10.88	78.71 78.39	469 473
	[2]	10.81 10.15 10.33	79.51 79.38 79.07	492 465
	[3]	11.13 10.81	79.95 80.11	460 493
	(B) [1]	11.16 10.57 10.72 10.82 10.70	78.68 79.03 79.07 79.86 79.64	474
	[2]	10.51 10.28 10.58 10.76	78.91 79.20 79.36 79.58	442 501

2. $C_{31\sim33}H_{50\sim54}O_4$

		H%	C%	mol. wt.
cal.		10.37~10.58,	76.54~77.04,	486~514
obs.	(A) [1]	10.33 10.15	77.21 76.98	512
	[2]	11.02 10.73	76.83 76.62	472
	[3]	10.55 10.22	77.20 77.53	461
	(B) [1]	10.97 10.65 10.70 10.74	76.66 76.92 77.05 76.74	507
	[2]	10.51 10.28 10.71	76.35 76.10 76.49	467

Then these formulas are resemble to those of the acidic sapogenins respectively, oleanolic acid $C_{30}H_{48}O_3$ or $C_{31}H_{50}O_3$ and hederagenin $C_{31}H_{50}O_4$. And if we add each one molecule of glucuronic acid, galactose, and rhamnose to this formula, by the following equation it is equal to the formula of each saponin.

$$[1]\quad (C_{30\sim32}H_{48\sim52}O_3 + C_6H_{10}O_7 + C_6H_{12}O_6 + C_6H_{13}O_5) - 3H_2O = C_{48\sim50}H_{77\sim81}O_{18}$$

$$[2]\quad (C_{31\sim33}H_{50\sim54}O_4 + \;''\; + \;''\; + \;''\;) - 3H_2O = C_{49\sim51}H_{79\sim83}O_{19}$$

(F) Acetyl derivatives of sapogenin :—

We obtain the following acetyl derivatives from each sapogenin by ordinary method.

[1] Diacetyl sapogenin. mel. pt. 175°. Needle crystal.

$C_{35}H_{54}O_5$. cal. H 9.83% C 75.81%, mol. wt. 554.
obs. H% 10.14 10.24, C% 75.36 75.77, mol. wt. (Rast) 549.
(titration) $226\times2=452$, $\times2.5=565$, $\times3=678$.

[2] Triacetyl sapogenin. mel. pt. 212°, Needle crystal.

$C_{38}H_{58}O_7$, cal. H 9.34%, C 72.84%, mol. wt. 626.
obs. H% 9.80 10.07, C% 73.33 73.67, mol. wt. (Rast) 637.
(titration) $193\times3=579$, $\times3.5=675$, $\times4=772$.

It is not explained why the results of the estimation of molecular weight by the titration deviate from the true value and what is the form of the remaining oxygen which is not carbonyl, but has been able to perform methylation by methyl-sulfate.

Summary.

(1) We have studied the crystalline saponin of soya bean which Y. Sumiki had investigated already, and have obtained the results that its molecular formula is $C_{48\sim50}H_{77\sim81}O_{18}$ and the products of hydrolysis are sapogenin ($C_{30\sim32}H_{48\sim52}O_3$), glucuronic acid, galactose, and rhamnose.

(2) Now we have isolated at the same time an amorphous saponin, of which molecular formula is $C_{49\sim51}H_{79\sim83}O_{19}$. Its sapogenin is $C_{31\sim33}H_{50\sim54}O_4$ and the others are identical with the former. Both have not haemolytic power.

(3) Each saponin is isolated five isomers according to the period of the hydrolysis and is required each mutual relation. And we have showed that the molecular formula of these neutral sapogenins are respectively resemble to those of the acidic sapogenins, oleanolic acid and hederagenin.

(4) If these saponins and sapogenins are heated with copper oxide or lead chromate, they liberate the considerable volume of methan or ethan gas. We have isolated the two more saponins that are water-soluble and haemolytic (crystal melts at 270° and amorphous 243°), of which we are to report later. Then we think that three kinds of saponin E. Walz showed are the following kinds of ours. One of them (mel. pt. 225°) is the crystalline saponin, ones (mel. pt. 280° hexagonal) is its natrium-salt and the remains (mel. pt. 272° haemolytic) are the saponin that is water-soluble.

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Studies on the Germination of Seeds. Part III.

Transformation of Carbohydrates During Germination of Soy-Bean Seeds.

By

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(Received November 18, 1933.)

With regard to the carbohydrates of the matured soj-bean seeds, Prof. M. Yukawa[1] made a very valuable investigation and found the following substances :—

Total carbohydrates (in anhydride form)			21.69%
Cane sugar	5.90%	Galactan	4.64%
Stachyose	3.52%	Fiber (crude)	3.85%
Araban	3.80%		

The present paper is to treat briefly the transformations of the foregoing substances, and also reducing sugar and starch, which appear in the seedlings of soy-beans during germination.

Method of Analysis.

Reducing sugar :— The samples were extracted with hot 90% alcohol, clarified with lead acetate, and estimated directly by Bertrand's method and expressed as glucose.

Cane sugar :— The samples were treated with alcohol and lead acetate as in the case of reducing sugar, and inverted with dilute HCl. Then, the total reducing power was determined. Cane sugar was calculated by subtracting from this value which the reducing sugar and the stachyose theoretically indicate.

Stachyose :— Stachyose was calculated from the difference between the weight of mucic acid obtained from all the specimens and that from the residues extracted with hot 90% alcohol.

Starch and dextrin :— Starch and dextrin were estimated together as they were difficult to separate. The residues extracted with hot 90% alcohol were digested with malt extract, and then the reducing power corresponding to starch and dextrin was determined in the usual manner.

Araban :— Araban was determined by Ôshima and Kondô's furfurol method[2]. Pentosan and methyl-pentose were also estimated by the same

method, but the amounts of these substances were rather insignificant and not worth mentioning.

Galactan :— Galactan was estimated by the mucic acid method adapted by H. D. Dore[3] from the residues extracted with hot 90% alcohol.

Crude fiber :— Crude fiber was determined as usual.

Results of Analysis.

White autumn seeds (larger specimens) of the bean produced in Korea were used as material for the experiment. The seeds, and the seedlings plucked out every 2, 5, 7, and 10 days after sowing in a dark room were analysed. The figures shown in the following table indicate the per cent of the original weights of the soj-bean seeds used :—

	Seeds	Seedling (days after sowing)			
		2	5	7	10
Reducing sugar	0.5	2.5	4.7	2.7	1.0
Cane sugar	5.0	3.1	1.9	0.8	0.5
Stachyose	3.7	2.4	0.8	0.5	0.4
Starch and dextrin	0.4	2.0	5.5	7.3	9.0
Araban	4.5	4.9	5.2	5.7	6.1
Galactan	4.9	4.0	3.5	3.1	2.9
Crude fiber	3.6	3.8	4.2	4.8	5.2
Total	22.6	22.7	25.8	24.9	25.1

Summary.

Reducing sugar, which is scarcely detected in the seeds, is increased the earlier stage of germination and decreased afterwards. Cane sugar and stachyose are decreased gradually. Starch and dextrin are markedly increased. Galactan is decreased; araban and crude fiber are increased. The total amounts of carbohydrates do not show any marked changes, during the development of the seedlings in the periods of the experiment.

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Über die Bildung des Harnstoffs aus Prolysin, Citrullin, verschiedenen Hydantoinen und aus Eiweisskörpern durch Einwirkung von Schwefelwasserstoff in schwach alkalischer Lösung.

Von

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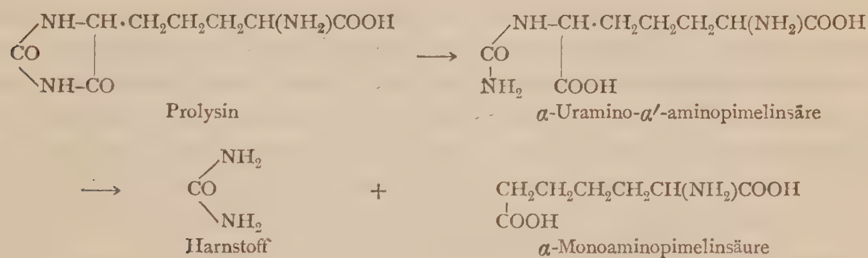
(Eingegangen am 1, November 1933)

In der vorigen Arbeit[1] hat Wada nachgewiesen, dass der Hydantoinring des Prolysins im Gegenwart von Bariumcarbonat durch Einwirkung von Schwefelwasserstoff in Harnstoff abgespalten wird und zwar nach folgendem Schema :—



Später wurde festgestellt, dass das Hydantoin des Prolysins, d. h. Pentamethylendihydantoin, Citrullin, sowie die Hydantoine der gewöhnlichen Aminosäuren wie Leucin, Phenylalanin usw. in analoger Weise unter Bildung des Harnstoffs gespalten werden.

Neulich ist es den Verfassern gelungen, die Bildung des Harnstoffs unmittelbar aus Eiweisstoff durch Einwirkung von Schwefelwasserstoff nachzuweisen. In diesem Fall wurde aber die Reaktion in verdünnter ammoniakalischer Lösung vorgenommen, um den Eiweisstoff in Lösung zu bringen.

Der gebildete Harnstoff wurde als freie Substanz oder als Nitrat, Oxalat, und Dixanthylderivat gereinigt und identifiziert. Da die Spaltung des Harnstoffs aus Prolysin oder Citrullin durch Schwefelwasserstoff fast quantitativ verläuft, so ist es möglich die Menge des Prolysins und Citrullins im Eiweissmolekül aus dem gebildeten Harnstoff zu ermitteln,

Experimenteller Teil.

1. a) 0,2 g Citrullin und 0,2 g Bariumcarbonat wurden in 20 ccm Wasser verteilt, 10 Minuten auf dem Wasserbad erwärmt und dann Schwefel-

1) Proc, **9** (1933), 43~46; Biochem. Zeitschr., **262** (1933) 57~67.

wasserstoff durchgeleitet. Die Uraminogruppe des Citrullins wird dadurch reduziert und spaltet Harnstoff ab. Wenn das Reaktionsgemisch mit Äther wiederholt extrahiert und der ätherische Extrakt verdampft wide, so erhält man den Harnstoff als Dixanthylverbindung vom Schmelzpunkt 260 bis 261°. Der oben erwähnte ätherische Extrakt gibt die P. Ehrlichsche sowie die Schiffsche Reaktion sehr stark. Durch Einwirkung von Urease in wässeriger Lösung wird Ammoniak gebildet.

b) 2 g Leucinhydantoin wurden in 50 ccm verd. Ammoniak (0.08 n) gelöst, 15 minuten auf dem Wasserbad erwärmt und in oben erwähnter Weise behandelt. In diesem Fall entsteht nebst Harnstoff Isobutylessigsäure, welche in wasser schwer lösliches, blaues Kupfersalz bildet. Im Kapillarrohr erhitzt, zersetzt sich das Kupfersalz bei 293°.

Analyse des Kupfersalzes von Isobutylessigsäure:

1.962 mg Subst.,	0,529 mg CuO,	Cu=21,27%.
Ber. für $(C_6H_{12}O_2)_2Cu$,		Cu=21,65%.

Zur quantitativen Bestimmung des gebildeten Harnstoffs wurden 0,0100 g Leucinhydantoin in 50 ccm einer mit Magnesiumoxyd gesättigten Lösung gelöst und Schwefelwasserstoff durchgeleitet. Man setzte nun soviel Essigsäure zu bis die Lösung gegen Phenolrot neutral reagierte, und nach dem Vertreiben des Schwefelwasserstoffs wurde die Lösung mit 1 ccm 10%iger Urease Lösung und wenig flüssigem Paraffin versetzt, und 15 Minuten auf dem Wasserbad bei 50° erwärmt um den gebildeten Harnstoff in Ammoniak zu verwandeln. Nach dem Erkalten wurde das gebildete Ammoniak mit Schwefelsäure in bekannter Weise titriert.

H_2SO_4 verbraucht: 1,4 ccm(1 ccm=0,004561 g H_2SO_4)=0,0063854 g H_2SO_4.
=39,0% Harnstoff.
Ber. für Leucinhydantoin =38,4% Harnstoff.

Man sieht, dass die Spaltung des Harnstoffs in diesem Fall fast quantitativ verlaufen ist.

c) 5 g Phenylalaninhydantoin wurden in 100 ccm verd. Ammoniak gelöst und genau in analoger Weise behandelt wie Leucinhydantoin. In diesem Fall bildet sich an Stelle der Isobutylessigsäure Phenylpropionsäure. Das Kupfersalz der letzteren kristallisiert glänzenden hellgrünlich blauen Nadeln. Es schmilzt bei 215° unter Zersetzung.

Analyse des Kupfersalzes von Phenylpropionsäure:

2,100 mg Subst.,	0,465 mg CuO,	Cu=17,69%.
Ber. für $(C_9H_9O_2)_2Cu$		Cu=17,57%.

2. Bildung des Harnstoffs aus Casein durch Einwirkung von Schwelwasserstoff in ammoniakalischer Lösung.

100 g Casein wurden in 200 ccm verd. Ammoniak (0.08 n) gelöst und

nach dem Zusatz von 800 ccm einer mit Magnesiumoxyd gesättigten Lösung 20 Minuten auf dem Wasserbad erwärmt und Schwefelwasserstoff durchgeleitet. Um den gebildeten Harnstoff von Eiweiss zu befreien, wurde die Lösung mit Essigsäure angesäuert, abfiltriert und eingedampft. Der dadurch zurückgebliebene Rückstand wurde mit Äther extrahiert. Beim Verdampfen des Äthers schied sich der Harnstoff als farblose Nadeln aus. Der letztere ist in Wasser und Alkohol löslich, schmilzt bei 132° und gibt die P. Ehrlichsche sowie die Schiffsche Reaktion sehr stark. Es wird durch Einwirkung von Urease in wässeriger Lösung in Ammoniak gespalten. Mit Xanthydrol bildet er Dixanthylharnstoff vom Schmelzpunkt 260 bis 261°. Die Ausbeute an Harnstoff betrug 1.8% des Caseins.

Analyse des Harnstoff:

2,104 mg Subst., 0,855 ccm N_2(758,5 mm 24°), N=46,54%.
Ber. für N_2H_4CO, N=46,66%.

Analyse des Dixanthylharnstoffs:

5,364 mg Subst., 0,303 ccm N_2(757,5 mm 24°), N=6,47%.
Ber. für $N_2H_2CO \cdot 2(C_{13}H_9O)$, N=6,66%.

Die Bestimmung des aus verschiedenen Eiweisskörpern gebildeten Hanstoffs wurde in analoger Weise ausgeführt wie bei Leucinhydantoin. In diesem Fall wurde aber die in Lösung gehaltenen Eiweisstoffe durch Einleiten des Schwefelwasserstoffs teilweise wieder gefällt und die Reaktion verlief nicht glatt, so dass ein viel niedrigerer Wert erhalten wurde als man erwartete. Im folgenden werden Resultate tabellarish zusammengestellt:

	gelöst in $NH_4OH+MgO$		Verbraucht H_2SO_4	= Harnstoff
(1) Edestin ……2,0535 g	50 ccm	30 ccm	17,9 ccm	2,43%
2,0225	〃	〃	17,6	2,43
(2) Zein ……1,0123	30	〃	1,9	0,52
0,9140	〃	〃	1,6	0,48
(3) Ovalbumin ……2,0490	〃	50	1,3	0,18
2,0138	〃	〃	1,2	0,16
(4) Glycinin ……0,7029 (extrahiert mit 0.2% NH_4OH)	50	20	1,9	0,75
(5) Oryzanin ……1,0246 (extrahiert mit 0.2% NH_4OH)	〃	〃	1,7	0,46
(6) Casein ……2,0296	〃	〃	13,6	1,88
2,0153	〃	〃	13,6	1,89
(7) Casein ……2,4251	〃	—	12,6	1,45
2,4342 (extrahiert mit 0.2% NaOH)	〃	—	14,0	1,51
(8) Gelatine ……3,0808	—	50	21,1	1,91
(9) Fibrin ……0,9176	50	〃	6,0	1,82

Da Zein kein Prolysin in seinem Molekül hat, so muss der Harnstoff ausschlizsslich aus Citrullin gebildet werden.

A Study on the Effect of Fatty Acids on Nutrition.

II.—Experiments with Diets composed of Rice, Oil and Lipoid containing Linoleic or Linolenic Acid.

By

Ume TANGE.

(Received August 23, 1933.)

When young rats were restricted to fat-free diets, they developed characteristic symptoms accompanying impairment of growth, denuded areas on skin and "scaly" condition of feet, and they were cured by the administration of either linoleic or linolenic acid(1). This observation led the author to further determination as to what function oils and phospholipins containing these essential acids play in animal physiology.

Recently Evans and Lepkovsky(2) indicated that the fatty acids from rice-starch were very potent in relieving disease in rats fed on a fat-free diet, while the fatty materials from potato-starch were ineffective. In our experiments, however, when potato-starch in the fat-free diet was replaced by either polished-rice powder or rice-starch, the growth of the animals was arrested and the fur was stained with blood, which was supposed to be bled around the nose and mouth. In this case, unfortunately, the sick animals were not cured with either linoleic or linolenic acid even though a high level of the yeast extract was administered, but the substitution of whole dried yeast resulted in a marked growth, recovering the ill conditions. This evidence suggested, therefore, the possibility of the presence in the yeast of other growth factors, not present in the yeast extract.(3)(4)

Experimental.

Preparation of Materials used in the Experiments.

a) Lecithin :— Purification of lecithin from "Soyalex".*

Two hundred grams of "Soyalex" were extracted with ether at room temperatur. Into the extract two volumes of pure acetone were added, and allowed to stand until a precipitate had settled out. This precipitated the phospholipins and left most of the fat and cholesterol in solution. The clear solution was then decanted from the precipitate, redissolved in ether and

* "Soyalex" (the crude lecithin prepared from soy-bean by the alcohol extraction method in the Central Institute of the South Manchurian Railway Company) was kindly furnished to the author by the Ohzeki Company in Tokyo.

reprecipitated, and this was repeated until the ethereal solution was entirely clear. By the addition now of three volumes of absolute alcohol to each volume of ether, nearly all of the cephalin was precipitated, but lecithin remained in solution. Into the filtrate a warm solution of $CdCl_2$ in 85% alcohol was added until no more precipitate formed. The lecithin-$CdCl_2$ precipitated out as white crystals. This was washed several times with absolute alcohol until the filtrate had become colourless. The lecithin-$CdCl_2$ was now suspended in 95% alcohol and decomposed with alcoholic saturated solution of $(NH_4)_2CO_3$. The filtrate was then evaporated under reduced pressure in CO_2 atmosphere, and the residue was treated with ether to remove the admixture of $(NH_4)_3CO_3$ and Cd salts. By the addition of acetone into the ether extract the lecithin precipitated out as a pale-yellow waxy substance. This was washed several times with acetone and dried in vacuum. About 40 g of lecithin were obtained, which had an iodine value of 75 (Wijs). The analysis of total N (Kjeldahl): 2.00% and P (molybdate-method): 4.31%, N/P: 1.08/1.00.

Separation of saturated and unsaturated fatty acids in lecithin.

Forty grams of lecithin obtained by the method mentioned above were heated on a water bath for 2∼3 hours with saturated $Ba(OH)_2$ solution, and the resulted Ba soaps were now decomposed with 10% HCl. By extracting the hydrolyzed liquid with ether, about 20 g of fatty acids were obtained, which had an iodine value of 108 (Wijs). The mixed acids were converted into lead salts by the usual method; the lead salts of the unsaturated fatty acids were removed by thorough extraction with ether. After separating the ether-soluble lead salts from the insoluble ones, each of them was hydrolyzed in ether medium with HCl. Thus, 5.0 g of saturated acids having m p 56∼57°C and 7.5 g of unsaturated acids having an iodine value of 130 (Wijs) were obtained.

b) Preparation of vitamin B_2:— The most potent concentrate of vitamin B_2 recorded in the literature seems to be that obtained by Narayanan and Drummond[(5)] by means of adsorption by fuller's earth from yeast extract of pH 0.1.

Two hundred grams of the baker's yeast powder were treated in like manner as described in a previous paper,[(1)] except with alcohol of 50% concentration. After extracting the concentrated yeast extract with ether to remove fat completely, a saturated solution of $Pb(C_2H_3O_2)_2$ was added into it. The lead acetate precipitate was decomposed by suspending it in warm water and slowly adding 10% H_2SO_4 with constant stirring until acid to congo-red, whereupon the lead sulphate was removed by filtration. In order to remove any trace of lead that might be present, H_2S was passed through the filtrate

to saturation and the whole allowed to stand for several hours. The reaction of the filtrate was then rendered approximately 0.9 N with H_2SO_4, being almost at pH 0.1, and treated with 3 g of fuller's earth for every 100 c.c of the filtrate.

The mixture was well stirred mechanically for 1/2 hour, then filtered and the earth was well washed with 0.9 N H_2SO_4. The filtrate and washing were similarly treated, a second time with 1.5 g of fuller's earth for each 100 c.c.

Thus, 35 g of the "activated" fuller's earth were obtained, adsorbing nearly 2.5% of the original yeast, and 0.5 g of the earth corresponded to 0.0715 g of adsorbed organic matter.

c) Neutral fatty oil from soy-bean oil:— This was made by dissolving soy-bean oil in a mixture of ether and alcohol (1 : 1) and by treating it with about 5% in excess of the amount of alcoholic KOH needed to neutralize the free acids in the oil. The mixture was occasionally shaken and allowed to stand for some hours at room temperature, then it was diluted with distilled water and extracted several times with ether. The ethereal solution was washed with distilled water until the filtrate showed no more alkaline reaction. After dehydrating with anhydrous Na_2SO_4, the solution was evaporated as completely as possible in a high vacuum in CO_2 atmosphere.

The neutral oil thus obtained consisted of some palmitin and stearin, most of the liquid fatty glycerides of soy-bean oil and also a very little amount of unsaponifiable substances. The saponification and iodine values are as follows:—

Oil	Acid value	Saponif. value	Iodine value
Original soy-bean oil	0.479	193	139 (Wijs)
Neutral fatty oil	——	196	139 (Wijs)

d) Saponification of cod-liver oil:— Fifty grams of cod-liver oil were introduced under constant stirring into 125 c.c of 20% methyl alcoholic KOH solution and left overnight at room temperature, then the hydrolyzed product was added into the alcoholic solution containing the calculated amount of $CaCl_2$ in order to convert the K-soap into Ca-soap. The precipitate was decomposed with dilute H_2SO_4, and the separated fatty acids were extracted with ether. The subsequent procedure was the same as described in the part of the neutral fatty oil from soy-bean oil. Thus a pale yellowish semi-solid substance having an indine value of 129 was obtained.

e) Rice-bran and chrysalis oils.†

Oil	Saponif. value	Iodine value
Rice-bran oil	185	107 (Wijs)
Chrysalis oil	194	115 (Wijs)

Tsujimoto[6] stated that fatty acids of rice-bran oil consisted of 20% palmitic acid, 45% oleic acid and 35% isolinoleic acid.

The analytical data of chrysalis oil by Kimura[7] are listed in the following Table:

Table I.

Acid value	Sap. value	Iod. value	Rhodan value	Unsap. matter %	Total fat %		
1.57	191.58	141.8	93.0	0.98	93.08		
					Saturat. acids %	Unsaturated acids %		
						Oleic acid	Linoleic acid	Linolenic acid
					23.9	22.2	27.3	26.6

Feeding of Animals.

Male albino rats of 40∼50 g were kept two or three in a cage with the various diets listed in Table II. All the diets were supplemented with the known necessary factors as follows: Vitamin A, D and B were supplied by administering biosterol*, irradiated ergosterol* and alcoholic extract of yeast or whole dried yeast respectively. Semi-solid oils were given by dissolving them in liquid paraffin; otherwise, the technique of feeding was the same as in the previous experiments.[1]

Table II. Composition (in g) of diets.

Diet	I (Fat-free diet)	II	III	IV	V	VI	VII	VIII	IX
Casein (fat-free)	20	20	20	18	18	18	18	20	20
Potato-starch (fat-free)	76							75	
McCollum salt mixture[8]	4	4	4	4	4	4	4	4	4
Polished-rice powder		76		75	75				75
Rice-starch			76						
Rice-bran				3					
Whole dried yeast					3				
Half-polished rice, retaining about 40% germ.						78			
Unpolished rice							78		
Lecithin								1	1

† I wish to thank Mr. Y. Kawakami of the Kao-Soap Company for funishing the oils.
* I wish to thank Dr. M. Sumi and Dr. J. Nakamiya for supplying the irradiated ergosterol and biosterol.

Results and Discussion.

a) The growth of rats receiving the polished rice (II) or the rice-starch diet (III) was very much inferior to those receiving the potato-starch diet (I). To our surprise the growth on the two former diets was retarded from the beginning, and the fur and the fore paws were mostly stained with red pigments like blood, and the abnormal condition of fur, giving an appearance of cotton, was often noticed (one of examples being illustrated by Photo.).

Following the bareness around the nose, the mouth and the eyes, there was a tendency to lose the hair on the whole body. The bald areas looked moist and inflamed, but showed no definite characteristic symptoms developed with the fat-free diet, namely the scurfy condition of the skin, "scaliness" of the feet and etc.

Photo.—Showing the onset of a characteristic symptom of a rat which had been fed with the polished-rice diet (Diet II).

The sick animals on these rice diets could not be recovered in spite of the increased use of the yeast extract, or cured by linoleic acid* unless the rice in the diets was replaced by potato-starch or the yeast extract by whole dried yeast. This fact called our attention to the possibility of the presence of toxic substance or substances in rice, which may be neutralized by whole dried yeast, because the substitution of it for the yeast extract brought about a marked improvement, showing resumption of weight and soft fine hair on the denuded areas.

It was an outstanding phenomenon that rats receiving the polished-rice diet in which 3% of rice-bran (IV) were added or those receiving the half-polished-rice diet (VI) developed the symptom resembling that on the polished-rice diet (II), though in the case with the half-polished-rice diet (VI) the rats showed very satisfactory growth (Charts 1∼3). The rats receiving the unpolished-rice diet (VII) attained a normal growth and appearance (Chart 4).

On autopsy of the rats fed with the rice diets, some lesion was often noticed in the liver and the kidney, sometimes exhibiting a white kidney.

b) Linoleic acid with vitamin B_2:— An attempt was made to determine whether linoleic acid could spare vitamin B_1, as a parallel experiment on vitamin B_2.(1) For this purpose the "activated" fuller's earth described previously in this paper was used as vitamin B_2 source.

* I desire to thank Dr. Y. Sahashi for generously supplying pure linoleic acid.

When fed with ration containing 0.5% of the "activated" fuller's earth, there appeared decline of weight as well as anorexia, and some died in spite of increase of the dose. Since the failure of growth and death were found due to an inadequate technique for the preparation of vitamin B_2, an autoclaved yeast extract[9] was employed instead of the "activated" fuller's earth; then there was a satisfactory growth for some intervals, but gradually the growth was impaired without any onset of the characteristic convulsion of vitamin B_1 deficiency. In this case, the administration of 2 drops daily of active oryzanin* induced a remarkable growth on the rats (Chart 5).

This evidence, therefore, led to conclude that linoleic acid had no "sparing" action on vitamin B_1 as in the case with B_2.[2]

c) Lecithin:— The rats reared on the diet containing 1% lecithin manifested an optimum growth; the fur was very fine and lustrous. In 1932, Trautman[10] stated that the feeding of 2 g daily of lecithin to a guinea pig accelerated growth, while 4 g retarded it. In this experiment, when given in the level of 1.5% of lecithin, growth was definitely improved but a proportion higher than 1.5% was not used since the pure lecithin was insufficient to ascertain Trautmann's experiment (Chart 6).

d) Neutral fatty oil of soy-bean oil:— Drummond and Gregory[9] have reported that the cause of the failure of the rats on the dietaries containing synthetic fat was due to a toxic substance, which was probably produced during the prolonged heating necessary for the esterification. This suggestion led the author to use the natural fatty oil free from fatty acids.

When 3 drops daily of the fatty oil were given the rats grew as healthy as those with 2 drops daily of linoleic acid itself. The oil had an effectiveness in alleviating the deficiency disease produced by the fat-free diet when the symptoms were not too advanced. It was, however, found that its curing power was less than that of linoleic acid itself (Charts 7 and 10).

e) Fatty acids of cod-liver oil:— As growth had been unsatisfactory on the diet containing 0.5% of the fatty acids, 2 drops daily of 30% of the acids dissolved in liquid paraffin were given, which exerted no appreciable influence on growth, and the animals had very similar symptoms described on the fat-free diet.[1] When a larger dose was given there was a tendency to produce diarrhea (Chart 8).

f) Chrysalis oil:— The group fed with 2 drops daily of the oil showed an excellent growth with a fine lustrous coating of hair. When the weight had exceeded 200 g the oil was replaced by 2 drops of 30% rice-bran oil dissolved in liquid paraffin, but the animals could continue growth until the

* I am indebted to Dr. S. Odake for supplying the active oryzanin which cured severe antineuritic pigeons with 4 mg daily.

experiment was terminated (Chart 9 A).

g) Rice-bran oil:— The feeding was started with the diet containing 1% rice-bran oil, but the animals soon lost their appetite and began to decline the weight, and there appeared the loss of hair around the nose and eyes. Though administered *per os* 2 drops daily of 30% oil dissolved in liquid paraffin or of chrysalis oil, no appreciable influence was exhibited either on growth or on malnutrition. When this condition continued, the animals have become emaciated and been unable to be relieved even though linoleic acid was administered, with the exception of one rat (Chart 9 B).

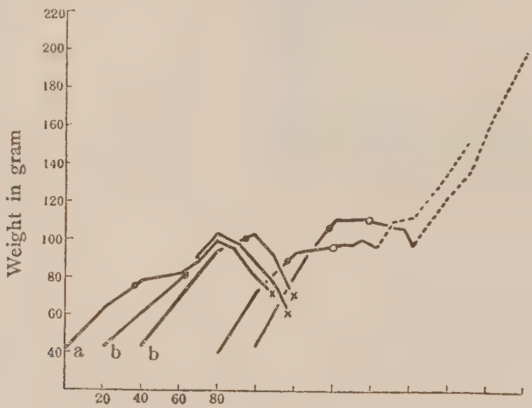
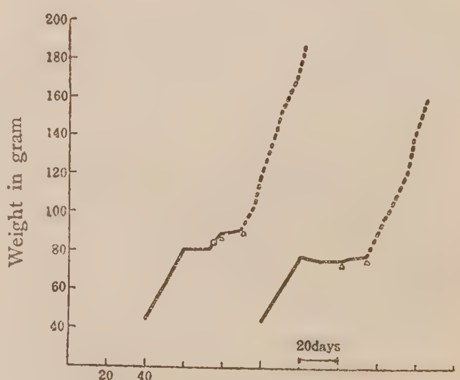


Chart 1.—Curves showing the growth of rats on Diet II before and after the substitution of whole dried yeast for the yeast extract, with administration of 2 drops daily of linoleic acid (a) or without the acid (b). The dotted line indicates the replacement of the yeast extract by whole dried yeast (Diet V), representing the prompt recovering of symptoms and resuming of weight. The small spot indicates an occurrence of bleeding and the small circle shows the sign ef losing hair on the body; × indicates death.

Chart 2.—Curves showing the growth of rats on Diet IV without administration of linoleic acid. During the period △—△ the yeast extract was furnished, and thereafter 3% whole dried yeast replaced it and at the same time the rice-bran was withdrawn. The small spot indicates an occurrence of bleeding.



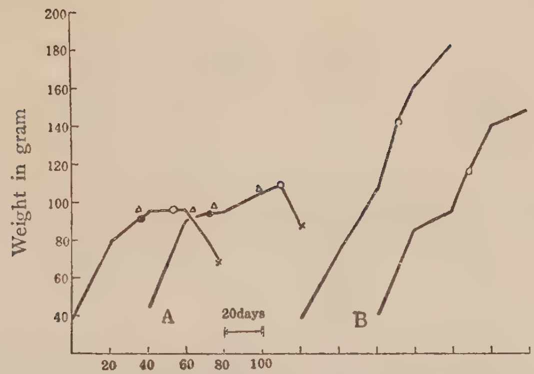


Chart 3.—Curves A showing the growth of rats on Diet III, supplemented with the yeast extract, but without linoleic acid. During the period △—△ a larger level of the yeast extract was employed, but showed no appreciable influence on growth. The small spot indicates an occurrence of bleeding and the small circle shows sign of bareness on the body; × indicates death.

Curves B showing the growth of rats on Diet VI without linoleic acid as well as the yeast extract. The small circle indicates an occurrence of losing hair on the body.

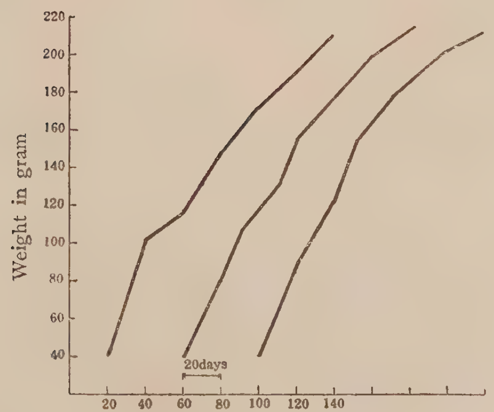


Chart 4.—Curves showing the growth of rats on Diet VII, without linoleic acid as well as the yeast extract.

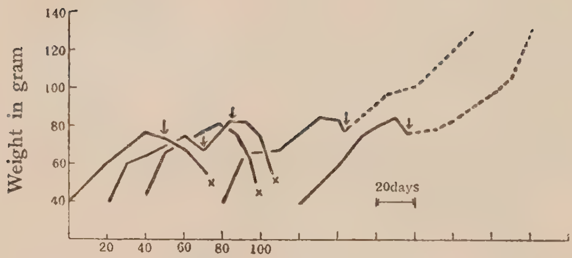


Chart 5.—Curves showing the growth of rats on Diet I, supplemented with "activated" fuller's earth or autoclaved yeast extract as vitamin B_2 source, and the renewed growth by supplying 2 drops daily of oryzanin solution as vitamin B_1. The arrow indicates adding of oryzanin solution; × indicates death.

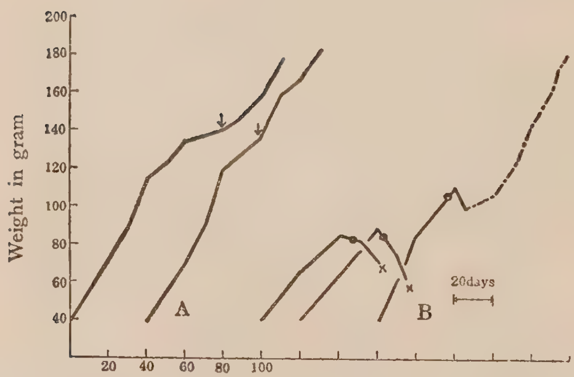


Chart 6.—Curves A showing the growth of rats on Diet VIII. The arrow indscates increase of lecithin to the level of 1.5%.

Curves B showing the growth of rats on Diet IX, and with the replacement of Diet VIII there appears the renewed growth and recovery from symytoms. The small spot indicates an occurrence of bleeding; × indicates death.

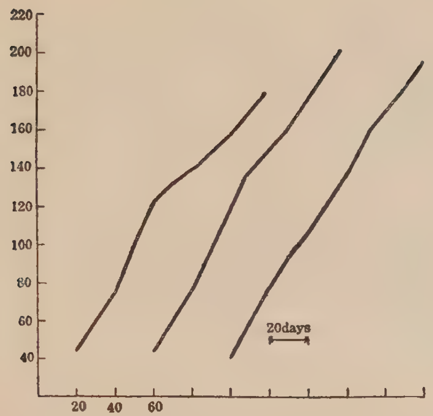


Chart 7.—Curves showing the growth of rats on Diet I, administered with 3 drops daily of neutral fatty oil of soy-bean oil.

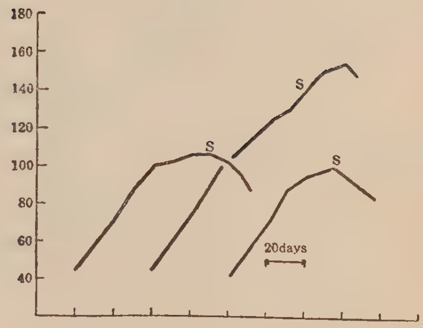


Chart 8.—Curves showing the growth of rats on Diet I, supplemented with 2 drops daily of fatty acids of cod-liver oil; s indicates "scaly" feet condition.

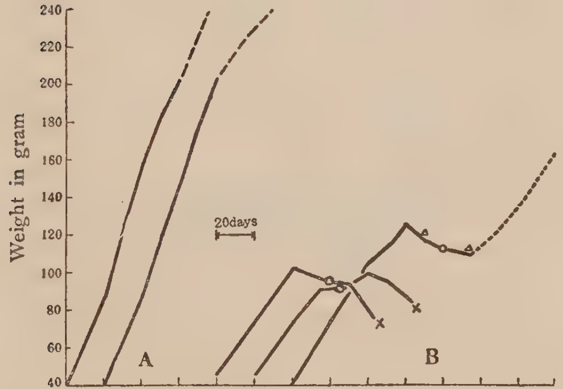


Chart 9.—Curves A showing the growth of rats on Diet I, administered with 2 drops daily of chrysalis oil. The broken line indicates the substitution of rice-bran oil for chrysalis oil.

Curves B showing the growth of rats Diet I, supplemented daily with 2 drops of rice-bran oil. During the period △—△ the oil was replaced by chrysalis oil. The dotted line shows the renewed growth and recovery from symptoms with administration daily of 2 drops of linoleic acid. The small circle indicates an occurrence of losing hair on the body; × indicates death.

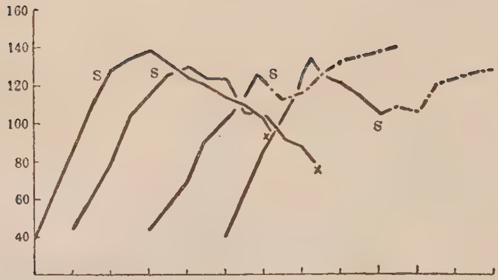


Chart 10.—Curves showing the growth of rats on Diet I, and renewed growth and slow recovery from the deficiency disease by treatment with 3 drops daily of fatty oil of soy-bean oil; s indicates "scaly" feet condition; × indicates death.

Summary.

(1) Growth and the general condition of animals are not attained by the diets containing 76∼78% of polished-rice powder, rice-starch, or half-polished rice as carbohydrates, except unpolished-rice diet, though an adequate amount of alcoholic yeast extract is provided. When, however, the yeast extract is replaced by whole dried yeast there appears a prompt recovery of the abnormal condition. The most characteristic phenomena are the occurrence of bareness on the body and of bleeding around the nose, the mouth, and the fore paws.

(2) Lecithin and soy-bean oil possess an almost equal nutritive value in animal physiology to linoleic acid.

(3) Chrysalis oil induces an excellent growth in rats, owing to its content of unsaturated fatty acids, namely linoleic and linolenic acids.

(4) The abnormal condition in nutrition of the rats fed on the rice-bran oil is not improved by administration of either chrysalis oil or linoleic acid. The cause of malnutrition and of the failure of growth with the former oil is not yet clear.

(5) The results of experiments with fatty acids prepared from cod-liver oil show that they cannot maintain the growth of animals and that the large dose causes a disturbance of the gastro-intestine.

(6) Linoleic acid is unable to "spare" either vitamin B_1 or B_2.

The author wishes to express her deep gratitude to Professor U. Suzuki for his many helpful suggestions throughout this work. She is indebted to the Keimei Society for financial assistance and is also grateful to Miss K. Miti for her willing help in feeding of the animals and preparing of the diets.

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